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SINGLE LABORATORY VALIDATION OF EPA METHOD 8140

by

Victoria Taylor
Dennis M. Hickey
Paul J. Marsden
S-CUBED
La Jolla, CA 92038-1620

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L. Don Betowski, Task Monitor

Environmental Monitoring Systems Laboratory
Office of Research and Development
U.S. Environmental Protection Agency
Las Vegas, NV 89114

ENVIRONMENTAL MONITORING SYSTEMS LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
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16. ABSTRACT

Method 8140 Organophosphorous Pesticides Parameters, of SW-846 was validated for use in the determination of 27 analytes in a single laboratory study. The method can be used for the analysis of 26 organophosphorous parameters in water, soil, and hazardous waste; the method is not suitable for monocrotophos because it showed poor recovery from soil and water. The performance of several GC columns, both packed and capillary, was established for the analysis of organophosphates. The use of megabore capillary GC columns over the use of packed columns specified in the current method is recommended for multiresidue organophosphorous analysis. Two phosphorus specific detectors, the flame photometric (FPD) and nitrogen phosphorus (NPD), were compared in terms of linearity, specificity, and sensitivity. Sample preparation and analysis techniques for organophosphates in aqueous and solid matrices were tested for ruggedness in order to identify critical method parameters. A proposed modification of Method 8140 is included together with accuracy and precision data for the determination of 26 method analytes in soil and water samples. Limited recovery data is also provided for additional organophosphorous compounds amenable to the method.

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FOREWORD

The Resource Conservation and Recovery Act of 1976 (RCRA) and its amendments require the U.S. Environmental Protection Agency (EPA) to regulate hazardous waste. Implementation and enforcement under RCRA requires analytical procedures that can provide data of known precision and accuracy on analytes in hazardous waste samples. Data collection under prescribed quality assurance/quality control procedures will allow the EPA to identify and delineate waste sites, to characterize waste composition, and to detect environmental contamination resulting from operations that generate hazardous wastes.

The document *Test Method for Evaluating Solid Waste*, Office of Solid Waste Manual SW-846[1], was published to provide a compilation of methodology to evaluate RCRA solid wastes for environmental and human health hazards. As part of the continuing efforts to improve analytical procedures, methods described in SW-846 are undergoing evaluation and validation. In 1986, S-CUBED was tasked to validate SW-846 Method 8140 for organophosphorous insecticides. The validation was conducted by using single-laboratory validation guidelines of the Association of Official Analytical Chemists (AOAC) Committee on Interlaboratory Studies and the EPA Environmental Monitoring Systems Laboratory, Las Vegas, NV.

ABSTRACT

Method 8140, *Organophosphorous Pesticides Parameters*, of SW-846 was validated for use in the determination of 27 analytes in a single laboratory study. The method can be used for the analysis of 26 organophosphorous parameters in water, soil, and hazardous waste. The method has not been validated for the determination of monocrotophos.The performance of a variety of GC columns, both packed and capillary, was established for the analysis of organophosphates. The use of megabore capillary GC columns is recommended for multiresidue organophosphorous analysis instead of the packed columns specified in the current method. Two phosphorous specific detectors, the flame photometric (FPD) and nitrogen-phosphorus (NPD), were compared to as to linearity, specificity, and sensitivity; and linearity plots for method analytes are included in the report. Sample preparation and analysis techniques for organophosphates in aqueous and solid matrices were tested for ruggedness in order to identify critical method parameters. A draft of a proposed modification of Method 8140 is included along with accuracy and precision data for the determination of 26 method analytes in soil and water samples.

This report was submitted in fulfillment of Contract No. 68-03-1958 by S-CUBED, A Division of Maxwell Laboratories, Inc., under the sponsorship of the U.S. Environmental Protection Agency. This report covers a period from February, 1986 to September, 1986, and work was completed as of February, 1987.

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ABBREVIATIONS AND SYMBOLS

DDVP	dichlorvos
EI	electron impact
EMSL-LV	Environmental Monitoring Systems Laboratory - Las Vegas
EPA	Environmental Protection Agency
EPN	santox
FPD	flame photometric detector
GC	gas chromatography
GPC	gel permeation chromatography
µg	microgram
µL	microliter
mL	milliliter
ng	nanogram
NPD	nitrogen phosphorous detector
RCRA	Resource and Conservation Recovery Act
RSD	relative standard deviation $RSD = \frac{SD}{\bar{X}}$
TEPP	tetraethylpyrophosphate
K-D	Kuderna-Danish

SECTION 1

INTRODUCTION

As part of its on-going Quality Assurance Program, the EPA tasked S-CUBED to conduct a complete single laboratory validation of Method 8140 of SW-846 for 27 organophosphorous analytes. The study addressed all aspects of Method 8140 as applied to water, soil, and hazardous waste. The results of the study demonstrate that Method 8140 is suitable for the multiresidue analysis of organophosphorous pesticides in environmental samples.

As written in SW-846, third edition[1], Method 8140 is applicable to the analysis of 21 organophosphorous parameters, with detection limits in the range of 0.1 to 5 μg/L for water. Method 8140 is a straightforward procedure in which samples are extracted with organic solvent, are concentrated, and are analyzed by GC with a phosphorous specific detector. Water samples are extracted by using one of two alternate procedures, Method 3510 (separatory funnel extraction) and Method 3520 (continuous liquid-liquid extraction). Two alternate sample preparation techniques are specified for soil samples and for non-aqueous hazardous waste samples, Method 3540 (soxhlet extraction) and Method 3550 (extraction by sonication). The gas chromatographic separation of organophosphates is accomplished by using three packed columns, 5% SP-2401 on Supelcoport, 3% SP-2401 on Supelcoport, or 10% SE-54 on Gas Chrom Q (Teflon column). Two alternate phosphorous specific GC detectors are recommended, the nitrogen phosphorus (NPD) or flame photometric (FPD). The use of two halogen sensitive detectors are allowed for chlorinated organophosphates: the microcoulometric (Coulson) detector or the electrolytic conductivity (Hall) detector.

This report includes a comparison of analyte recovery by using the sample preparation techniques specified for water and soil, an evaluation of several packed and capillary columns with retention times for organophosphates of regulatory interest, and a comparison of linearity, sensitivity, and detection limits for NPD and FPD detectors. As the current method allows for sample cleanup but does not present specific methodology, a study of the applicability of cartridge cleanup techniques for Method 8140 is also presented. Results of three ruggedness tests applied to sample preparation and analysis techniques are included along with critical method criteria. Method bias and precision was established by conducting recovery studies of method parameters spiked at multiple concentration into each sample matrix.

Based on the results of the validation study, a modification of Method 8140 is proposed. This revision of Method 8140 is a more efficient and comprehensive multiresidue technique for determination of organophosphorous insecticides in environmental matrices than the present method. This improvement in method performance is achieved primarily by improved chromatography of the method analytes. In the revised method, dual megabore capillary analysis is recommended which uses a polar DB-210 column (or equivalent) as the primary column and an SPB-608 column (or equivalent) as a confirmatory column. Sample preparation techniques are essentially the same as those specified in the present method except that 1:1 methylene chloride/acetone is recommended as the extraction solvent for solid samples.

SECTION 2

CONCLUSIONS

Method 8140 of SW-846 was applied to the determination of 27 organophosphorous analytes in water and soil. The method has been validated for use with 26 of these compounds. Because there were shortcomings in the present method in the area of GC analysis, a modified method has been prepared and is presented in Appendix A. Method detection limits for this modified method are presented in Table 1. The new version of Method 8140 allows the use of megabore capillary columns which provide the laboratory with resolution similar to that which can be achieved narrow bore capillary columns along with column capacity closer to packed column. This modified method represents improvement over the older technique and can be used to generate data of good quality with excellent repeatability.

Method 8140 has been ruggedness tested, and the critical method parameters have been identified. The proposed method includes a requirement to use a solvent mixture with a water miscible solvent for the extraction of solids (1:1 methylene chloride/acetone suggested) and a requirement to transfer concentrated extracts by using a solvent mixture containing some water miscible solvent. The recovery studies reported here demonstrate that Method 8140 has the accuracy, precision, and ruggedness to make it suitable for the routine analysis of 26 organophosphorous compounds in water, soil/sediment, and hazardous wastes. Application of standard methods of sample preparation failed to produce acceptable recoveries for monocrotophos; therefore, Method 8140 has not been validated for use in determining this compound. The method also has the potential to be used for other organophosphates besides the 26 validated in this study.

TABLE 1. METHOD 8140 DETECTION LIMITS USING FPD

Compound	Water (3510) (μg/L)	Soil (3540) (μg/kg)
Azinphos methyl	0.10	5.0
Bolstar (Suprophos)	0.07	3.5
Chlorpyrifos	0.07	5.0
Coumaphos	0.20	10.0
Demeton, O,S*	0.12	6.0
Dimethoate	0.26	13.0
Diazinon	0.20	10.0
Dichlorvos	0.80	40
Disulfoton	0.07	3.5
Ethoprop	0.20	10.0
EPN	0.04	2.0
Fensulfothion	0.08	4.0
Fenthion	0.08	5.0
Malathion	0.11	5.5
Merphos	0.20	10.
Mevinphos	0.50	25.
Naled	0.50	25.
Parathion, ethyl	0.06	3.0
Parathion, methyl	0.12	6.0
Phorate	0.04	2.0
Ronnel	0.07	3.5
Sulfotep	0.07	3.5
TEPP**	0.80	40
Tetrachlorovinphos	0.80	40
Tokuthion (Protothiofos)**	0.07	5.5
Trichloronate**	0.80	40

*Standard supplied as mixture of two isomers.

**Purity of these standards not established by the EPA Pesticides and Industrial Chemicals Repository, RTP, NC.

SECTION 3

RECOMMENDATIONS

Based on results obtained in the validation study of Method 8140, a modified method has been written and is presented in Appendix A. The specific changes that are suggested in the method are described below.

SAMPLE EXTRACTION

The modified Method 8140 requires that aqueous samples be extracted by using methylene chloride and that solids be extracted by using a 1:1 methylene chloride/acetone solvent mixture. A caution has been added that the analyst should use a drying agent only if necessary, since single laboratory results indicate that some organophosphate analytes are lost during drying with sodium sulfate. Analysts are also given advice to the effect that in single laboratory testing, better recoveries were obtained by using a separatory funnel for waters and soxhlet extractions for solids. In this way the laboratory can make an intelligent choice concerning whether they require the higher sample throughput that can be achieved by using sonication and continuous liquid-liquid extraction or if they need slightly more accurate results that can be obtained by using separatory funnel extraction or soxhlet extraction.

The laboratory is allowed to use either Kuderna-Danish (K-D) or rotary evaporation to reduce the volume of sample extract. Analysts are cautioned that samples should not be transferred with 100 percent hexane during sample workup as the more water soluble organophosphates (dichlorvos, fensulfothion, and dimethoate) may be lost.

SAMPLE CLEANUP

No sample cleanup is required for Method 8140, nor was one validated for its use. Instead, the laboratories are given guidance that one of several standard cleanup options may be used if particular samples require it. These options are Florisil chromatography, acid washed silica chromatography, or Diol cartridge cleanup. None of these cleanup methods have been specifically validated for Method 8140. Therefore, the laboratory is required to demonstrate greater than 70 percent recovery for any analytes to be determined by using a particular cleanup method. A special caution on the use of GPC is included.

GC ANALYSIS

The major change in this modified version of Method 8140 is in the type of GC columns to be used in the analysis of organophosphates. The use of megabore capillary analysis is now recommended in the method instead of packed column analysis. This modification became necessary because packed columns lacked the resolution to separate the full complement of method analytes. Three packed columns (3% SP-2401, 5% SP-2401, and 4% OV-210/6% SP-2100) are listed in the method with the caution that these columns can only be used if the method is to be used to determine a limited number of compounds. The use of two megabore capillary columns is strongly recommended, a polar column (DB-210 or equivalent) as a primary column and a moderate polarity column (the SP-608 or equivalent) as a secondary column.

SECTION 4

MATERIALS AND METHODS

EXPERIMENTAL DESIGN

The general procedures for single laboratory testing are outlined in *Validation of Testing/Measurements Methods*, EPA 600/X-83-0060.[2] They include a demonstration of:

- Method ruggedness.
- Method accuracy as determined by analysis of spiked samples.
- Method precision as determined by analysis of replicate samples.
- Upper and lower concentration limits outside which the method is no longer applicable.
- Method suitability for the matrix to be tested.

These guidelines were applied in the validation and testing of Method 8140. The statistical design of Youden and Steiner for ruggedness testing[3] was applied to sample preparation and analysis techniques. Both the method accuracy and method precision were determined by analysis of replicate spiked samples. Comparison of spiked sample results with the calculated amount of analyte spiked or *true* value was used to determine the method accuracy or bias. The relative standard deviation (RSD) of the replicate determinations was used to measure the single laboratory precision of the method.

Because the upper and lower concentration limits to which the method may be applied are primarily a function of the analytical system employed, the linearity and reasonable limits for both the NPD and FPD were established. The suitability of several packed and capillary columns was evaluated with both detectors for use in the analysis of multiresidue samples.

The final portion of the laboratory validation was a performance test of the method to determine its suitability for the analysis of organophosphate insecticides spiked into hazardous waste samples.

METHOD ANALYTES

The names, synonyms, and chemical structures for the 27 analytes for which modified Method 8140 was fully tested are given in Table 2. Table 3 presents physical data and acute LD_{50} values. Some compounds have up to three names listed. This is because besides the correct chemical name, the manufacturer of an agricultural chemical assigns a shortened name derived from the chemical name as well as a trade name. Also presented are the structures, chemical names, boiling or melting points, water solubilities and acute oral toxicities for each compound. The boiling point, melting points, water solubilities, and acute oral toxicities were taken from *The Pesticide Manual*[4]. Experimental details of how they were determined and additional references may be found there.

Standards for 25 of the analytes used in this study were provided by the Pesticides and Industrial Chemicals Repository of the EPA at Research Triangle Park, NC. Additional standards for tokuthion and trichloronate were provided as gifts by the Mobay Chemical Company, Kansas City, KS.

TABLE 2. NAMES AND CHEMICAL STRUCTURES OF PROPOSED METHOD 8140 ANALYTES[4,5,6]

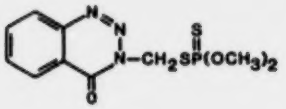
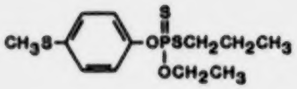
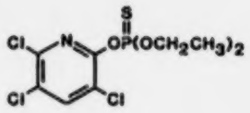
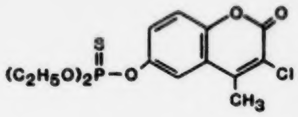
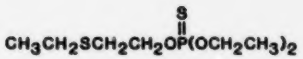
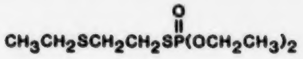
Common Name	Chemical Name	Structure
Azinphos methyl	*O,O*-dimethyl *S*-(4-oxo-1,2,3-benzotriazin-3(4H)-yl) methyl phosphorodithioate	
Bolstar	*O*-ethyl *O*-4-methylthiophenyl *S*-propyl phosphorodithioate	
Chlorpyrifos	*O,O*-diethyl *O*-3,5,6-trichloro-2-pyridenyl phosphorothioate	
Coumaphos	*O*,-3-chloro-4-methyl-2-oxo-2H-1-benzopyran-7-yl *O,O*-diethyl phosphorothioate	
Demeton O,S	*O,O*-diethyl *O*-ethylthioethyl phosphorothioate	 $CH_3CH_2SCH_2CH_2O\overset{S}{\overset{\|}{P}}(OCH_2CH_3)_2$
	O,O-diethyl *S*-ethylthioethyl phosphorodithroate	 $CH_3CH_2SCH_2CH_2S\overset{O}{\overset{\|}{P}}(OCH_2CH_3)_2$

TABLE 2. NAMES AND CHEMICAL STRUCTURES OF PROPOSED METHOD 8140 ANALYTES[4,5,6] (continued)

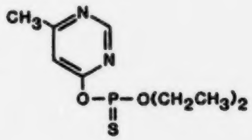
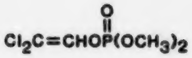
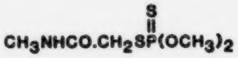
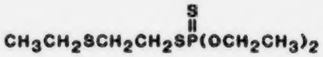
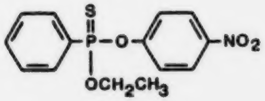
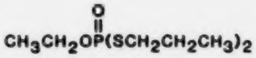
Common Name	Chemical Name	Structure
Diazinon	*O,O*-diethyl *O*-2-isopropyl-6-methylpyimidin-4-yl phosphorothioate	
Dichlorvos	*O*-2,2-dichloroethenyl *O,O*-dimethyl phosphate	 $Cl_2C{=}CHO\overset{O}{\overset{\parallel}{P}}(OCH_3)_2$
Dimethoate	*O,O*-dimethyl *S*-methyl carbamoylmethyl phosphorodithioate	 $CH_3NHCO.CH_2S\overset{S}{\overset{\parallel}{P}}(OCH_3)_2$
Disulfoton	*O,O*-diethyl *S*-2-ethylthioethyl phosphorodithioate	 $CH_3CH_2SCH_2CH_2S\overset{S}{\overset{\parallel}{P}}(OCH_2CH_3)_2$
EPN	*O*-ethyl *O*-4-nitrophenyl phenylphosphonothioate	
Ethoprop	*O*-ethyl *S,S*-dipropyl phosphorodithioate	 $CH_3CH_2O\overset{O}{\overset{\parallel}{P}}(SCH_2CH_2CH_3)_2$

TABLE 2. NAMES AND CHEMICAL STRUCTURES OF PROPOSED METHOD 8140 ANALYTES[4,5,6] (continued)

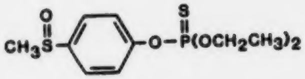
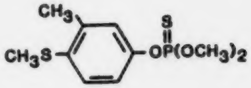
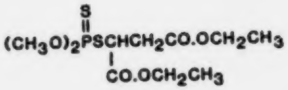
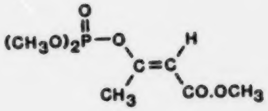
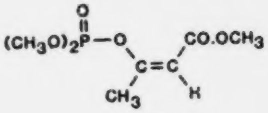
Common Name	Chemical Name	Structure
Fensulfothion	*O,O*-diethyl *O*-4-methylsulphinylphenyl phosphorothioate	
Fenthion	*O,O*-dimethyl *O*-3-methyl-4-(methylthio) phenyl	
Malathion	*O,O*-diethyl *S*-1,2-di(carboethoxy)ethyl phosphorodithioate	 $(CH_3O)_2\overset{S}{\overset{\|}{P}}S\underset{CO.OCH_2CH_3}{\underset{\mid}{C}}HCH_2CO.OCH_2CH_3$
Merphos	Tributyl phosphorotrithioite	$(CH_3CH_2CH_2CH_2S)_3P$
Mevinphos	*O,O*-dimethyl-1-carbomethoxy-1-propen-2-yl phosphoroate	 (E)  (Z)

TABLE 2. NAMES AND CHEMICAL STRUCTURES OF PROPOSED METHOD 8140 ANALYTES[4,5,6] (continued)

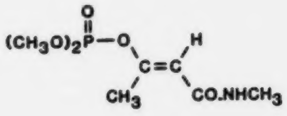
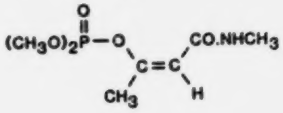
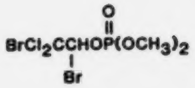
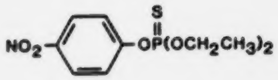
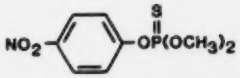
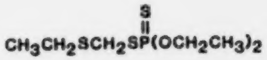
Common Name	Chemical Name	Structure
Monocrotophos	*O,O*-dimethyl-*O*-(Z)-1-methyl-3-(methylanimo)-3-oxo-1-propenyl phosphoroate	 (E)  (Z)
Naled	*O*-1,2-dibromo-2,2-dichloroethyl *O,O*-dimethyl phosphoroate	 $BrCl_2CCHOP(O)(OCH_3)_2$
Parathion, ethyl	*O,O*-diethyl *O*-4-nitrophenyl phosphorothioate	
Parathion, methyl	*O,O*-dimethyl *O*-4-nitrophenyl phosphorothioate	
Phorate	*O,O*-diethyl *S*-ethylthiomethyl phosphorodithioate	 $CH_3CH_2SCH_2SP(S)(OCH_2CH_3)_2$

TABLE 2. NAMES AND CHEMICAL STRUCTURES OF PROPOSED METHOD 8140 ANALYTES[4,5,6] (continued)

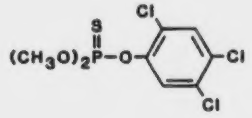
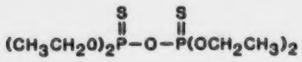
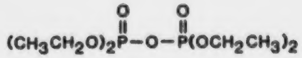
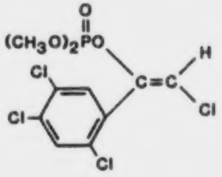
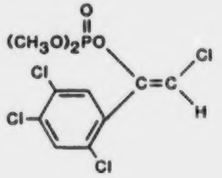
Common Name	Chemical Name	Structure
Ronnel	*O,O*-dimethyl *O*-2,4,5-trichlorophenyl phosphorothioate	
Sulfotep	*O,O,O',O'*-tetraethyl dithiopyrophosphate	 $(CH_3CH_2O)_2\overset{\overset{S}{\|}}{P}-O-\overset{\overset{S}{\|}}{P}(OCH_2CH_3)_2$
TEPP	*O,O,O',O'*-tetraethyl pyrophosphate	 $(CH_3CH_2O)_2\overset{\overset{O}{\|}}{P}-O-\overset{\overset{O}{\|}}{P}(OCH_2CH_3)_2$
Tetrachlorovinphos	*O*-(Z)-2-chloro-1-(2,4,5-trichlorophenyl)ethenyl *O,O*-dimethyl phosphoroate	 (E)  (Z)

TABLE 2. NAMES AND CHEMICAL STRUCTURES OF PROPOSED METHOD 8140 ANALYTES[4,5,6] (continued)

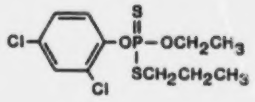
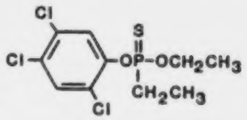
Common Name	Chemical Name	Structure
Tokuthion	*O*-2,4-dichlorophenyl *O*-ethyl *S*-propyl phosphorodithioate	
Trichloronate	*O*-ethyl *O*-2,4,5-trichlorophenyl ethylphosphonothioate	

TABLE 3. PHYSICAL DATA OF PROPOSED METHOD 8140 ANALYTES[4,5,6]

Common Name	Alternate Name	Acute LD_{50} (Rats) mg/kg	Boiling Point (°C)	Aqueous Solubility (mg/L)
Azinphos methyl	Guthion	16.4 (F)	73-74 (m.p.)	33 (20°C)
Bolstar	Sulprophos	304 (M)	210 (NA)	<5 (29°)
Chlorpyrifos	Dursban	135-163	42-43 (m.p.)	2 (25°)
Coumaphos	--	NA	NA	NA
Demeton O,S	Syotox	6-12 (M) 2.5-4 (F)	123/128 (1 mmHg)	60 (RT)
Diazinon	Spectracide	300-400	83-84 (0.0002 mmHg)	40 (20°C)
Dichlorvos	DDVP	56-108	35 (0.05 mmHg)	10×10^3 (20°C)
Dimethoate	Cygon	500-600 (M) 570-680 (F)	51-52 (m.p.)	25×10^3 (21°C)
Disulfoton	--	2.6-12.0	62 (0.01 mmHg)	25 (22°C)
EPN	--	33-42 (M)	36 (m.p.)	Insoluble
Ethoprop	Mocap	62 (Albino)	86-91 (0.2 mmHg)	750 (NA)
Fensulfothion	Dasanit	4.7-10.5 (M)	131-141 (0.01 mmHg)	1.54×10^3 (25°C)
Fenthion	Baytex	190-315 (M) 245-615 (F)	87° (0.01 mmHg)	2 (20°C)
Malathion	--	2800	156-157 (0.7 mmHg)	145 (RT)
Merphos	Folex	1272 (Albino)	115-134 (0.08 mmHg)	Slightly soluble
Mevinphos	Phosdrin	3-12	99-103 (0.3 mmHg)	Completely soluble
Monocrotophos	Azodrin	14	125 (0.0005°C)	1×10^6 (20°C)
Naled	Dibrom	430	110 (0.5 mmHg)	Insoluble
Parathion, ethyl	--	13 (M) 3.6 (F)	157-162 (0.6 mmHg)	24 (25°C)
Parathion, methyl	--	14 (M) 24 (F)	35-36 (m.p.)	55-60 (25°C)
Phorate	Thimet	1.6-3.7	118-120 (0.8 mmHg)	50 (RT)
Ronnel	Fenchlorophos	NA	NA	NA
Sulfotep	Bladafum	5	136-139 (2 mmHg)	25 (RT)
TEPP	--	1.12	124 (1 mmHg)	Miscible
Tetrachlorovinphos	Gardona	4000-5000	95-97 (m.p.)	11 (20°C)
Tokuthion	Protothiophos	925-966 (M)	125-128 (0.1 mmHg)	1.7 (20°C)
Trichloronate	Agritox	16-37.5	108 (0.01 mmHg)	50 (20°C)

NA = Not available
RT = Room temperature

SECTION 5

EXPERIMENTAL PROCEDURES

EVALUATION OF METHOD PROCEDURES

Method 8140 specifies the use of gas chromatography (GC) for the separation and quantitative determination of organophosphorous compounds using single phase packed columns. In recent years such columns have been replaced by mixed phase packed or capillary GC columns for the analysis of pesticide residues in environmental samples.[7,8] The initial effort in this validation study was to investigate the separation of organophosphates by using a variety of different GC columns.

Gas Chromatographic Column Evaluation

Packed Column Analysis–

Method 8140 currently specifies three packed column options. Two of the columns are a five percent and a three percent loading of SP-2401 on 100/120-mesh Supelcoport in 180 cm x 2 mm glass column. The third column is a 15 percent SE-54 on Gas Chrom Q (100/120 mesh) packed in a 50-cm x 1/8-inch Teflon column. The 5% SP-2401 column and three additional packed columns were evaluated as part of this validation study (Table 4). The other packed columns were a 6% OV-210/4% SP-2100 as suggested by Chau and Luke[7,9]; a 10% DEGS (recommended by Greg Doose of the Los Angeles Office of the U.S. Federal Department of Agriculture); and a 10% SP-2330 (equivalent McReynolds constants to DEGS but with a higher temperature limit[10]). All columns were 180 cm x 2 mm ID, with 100/120-mesh Supelcoport used as packing support. The SE-54 Teflon column is a volatile nerve agent column[11] and was not tested in this study because it could not be used to analyze late eluting compounds because of the low temperature limit for Teflon columns.

The 6% OV-210/4% SP-2100 and the 5% SP-2401 were found to be about equivalent for organophosphorous analysis after the separation of analytes was optimized by the use of temperature programming. Sample chromatograms for each column are presented in Figures 1 and 2. The retention times for compounds in these chromatograms are longer than the values in Table 4 because a slower column flow rate (20 mL/minute) was used when the sample chromatograph was run. It was found that a faster column flow (30 mL/minute) gave adequate separation of all analytes and retention times using 30 mL/minute are reported in Table 4. We judged the OV-210/SP-2100 column to be a somewhat superior column because it separated malathion, methyl parathion, and tokuthion while they co-chromatographed on the SP-2401. Table

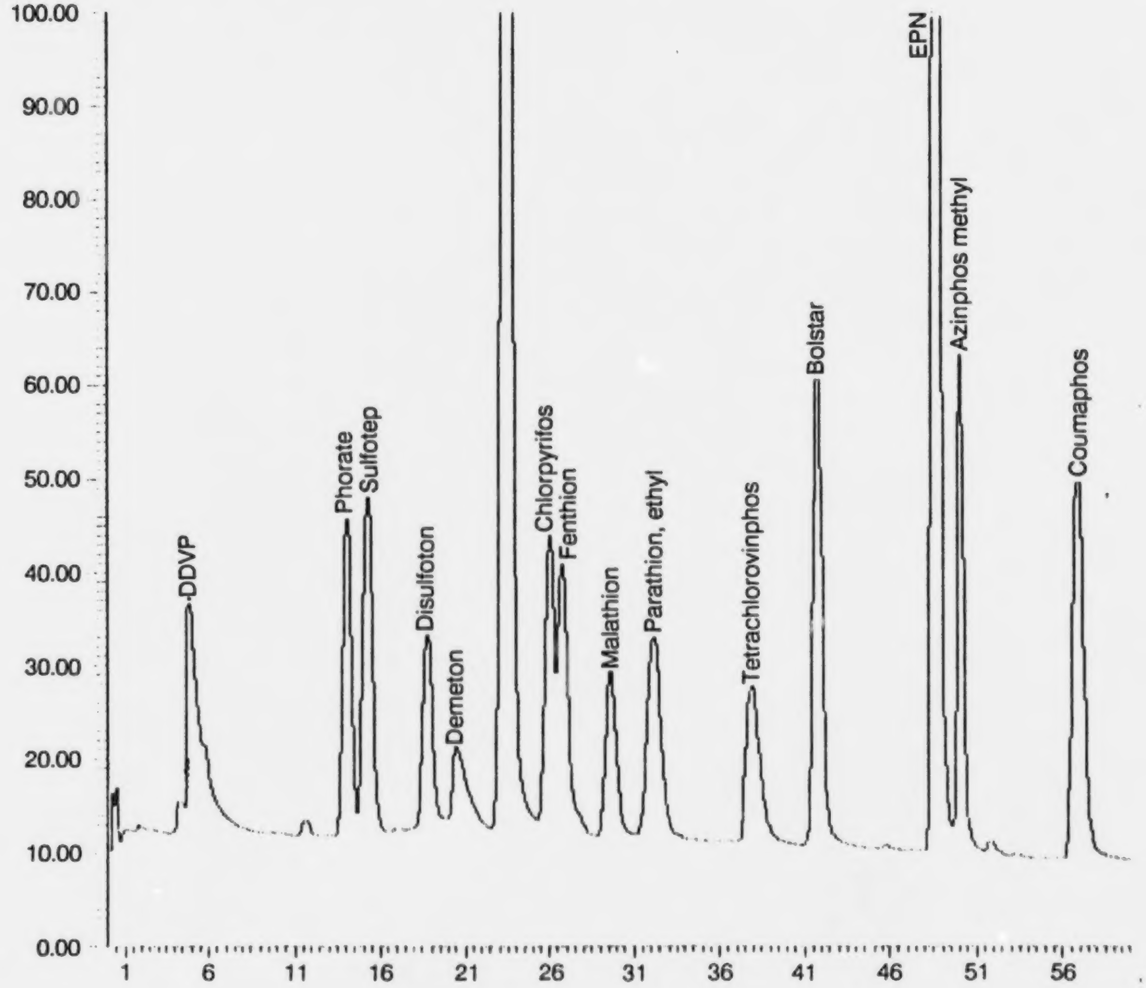


Figure 1. Fifteen organophosphorous insecticides separated on a 6% OV-210/4% SP-2100 packed column.

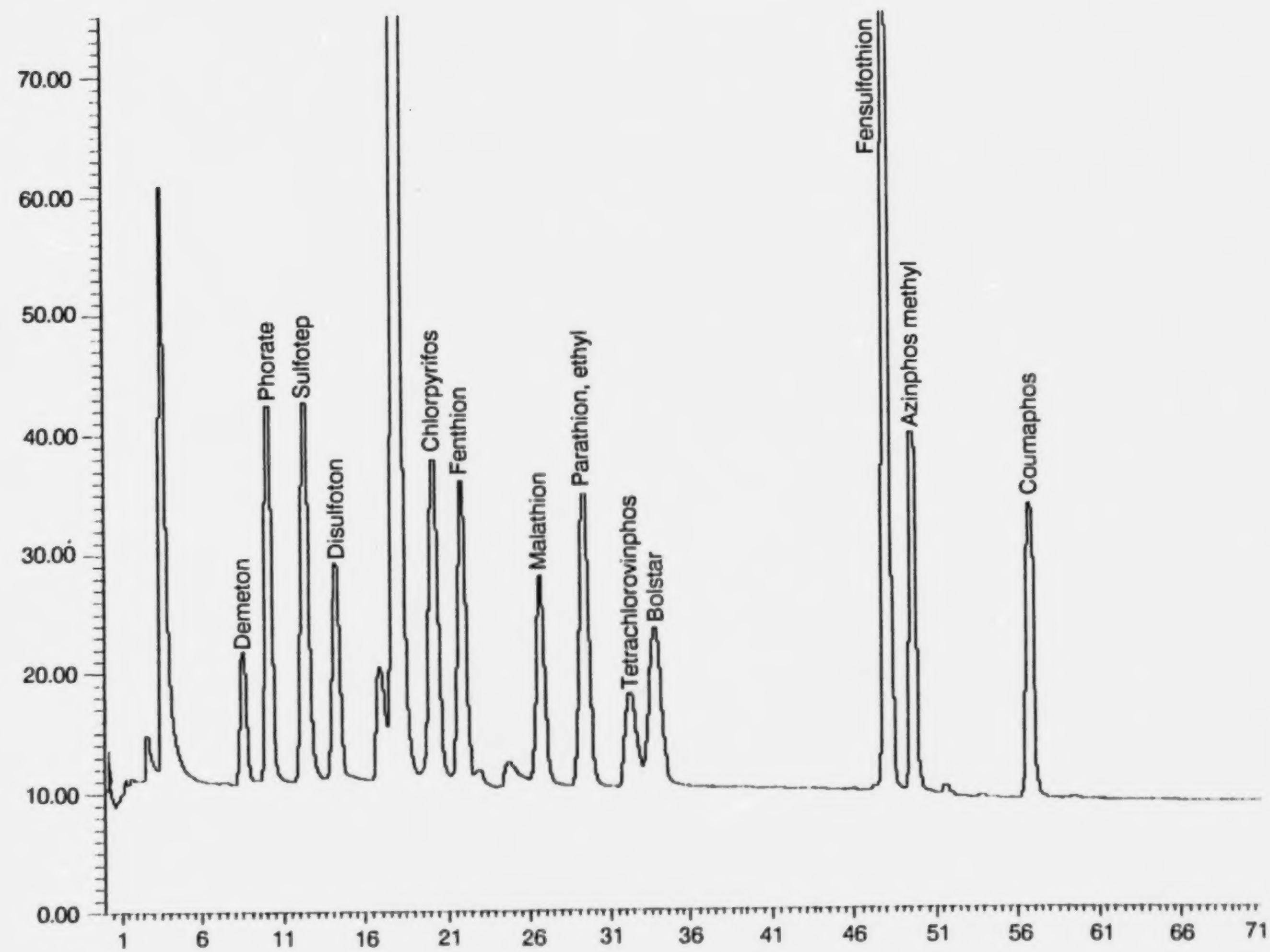


Figure 2. Fourteen organophosphorous insecticides separated on a 5% SP-2401 packed column.

TABLE 4. RETENTION TIMES FOR METHOD 8140 ORGANOPHOSPHOROUS PARAMETERS ON RECOMMENDED PACKED COLUMNS

Compound	6% 0V210/4% SP2100	5% SP2401
Azinphos methyl	45.91	35.67
Bolstar	31.96	25.39
Chlorpyrifos	17.58	14.37
Coumaphos	57.00	42.73
Demeton	13.67	12.79
Dimethoate	ND	ND
Diazinon	12.80	10.47
Dichlorvos	4.98	3.88
Disulfoton	13.26	11.27
Ethoprop	ND	ND
EPN	43.07	34.39
Fensulfothion	42.10	34.58
Fenthion	18.13	15.26
Malathion	20.44	18.67
Merphos	18.31	19.53
Mevinphos	9.72	8.56
Monocrotophos	18.77	16.36
Naled	11.39	10.09
Parathion, ethyl	23.19	21.49
Parathion, methyl	19.16	18.00
Phorate	10.81	8.86
Ronnel	15.84	13.24
Sulfotep	11.41	10.19
TEPP	3.56	3.05
Tetrachlorvinphos	28.21	24.29
Tokuthion	24.58	18.18
Trichloroate	ND	ND

ND = Not determined

Initial Temperature	130°C	130°C
Initial Time	3 minutes	3 minutes
Program 1 Rate	5°C/minute	5°C/minute
Program 1 Final Temperature	180°C	180°C
Program 1 Hold	10 minutes	10 minutes
Program 2 Rate	2°C/minute	2°C/minute
Program 2 Final Temperature	250°C	250°C
Program 2 Hold	15 minutes	15 minutes

4 summarizes the packed column retention times for 24 of the 27 method parameters on these two packed columns. Retention times for three compounds were not included because standards were not available at the time of the packed column evaluation.

The two columns with the highest liquid-phase loading, 10% SP-2330 and 10% DEGS, were found to be unsuitable for multiresidue organophosphorous analysis. The SP-2330 column failed because several of the organophosphate insecticides under consideration, most notably those with aromatic functional groups, did not elute from the column. While the DEGS column provided excellent resolution of the more volatile analytes, it failed because high boiling compounds (azinphos methyl, coumaphos, and EPN) could not be analyzed within a run time of less than two hours. The limitation of DEGS to the less volatile organophosphates is caused by its upper temperature limit of 200°C and has been reported previously.[12]

GC analysis using a 5% SP-2401 or 6% OV-210/4% SP-2100 with temperature programming is adequate for the analysis of most organophosphate insecticides; however, its inherent usefulness is limited by the low column efficiency of packed columns.[13] Neither the DEGS nor the SP-2330 column is recommended for multiresidue organophosphate analysis.

Capillary Column Analysis--

Three fused silica megabore capillary columns were evaluated: the DB-5, DB-210, and SPB-608. Megabore capillary columns are superior to packed columns because of their high efficiency. Megabore columns have greater sample capacity than narrow bore columns and are therefore less prone to inadvertent overloading than 0.25 - 0.32 mm i.d. fused silica capillaries. Using a glass sleeve adapter, megabore capillary columns can be mounted in regular quarter-inch injector ports without additional hardware other than a makeup gas connection for the detector.

The separation of organophosphates on two of the three megabore capillary columns evaluated was superior to the use of packed columns. Because the DB-5 failed to separate a number of compounds including fenthion, malathion, ethyl parathion, and chlorpyrifos, it is not recommended as a column for this method. The other two capillary columns gave much better separation of the method parameters. The DB-210 could be used to separate the greatest number of compounds; however, the DB-210 column is subject to oxidation, and care must be taken to limit its contact with air. The sample chromatograms in Figures 3 and 4 show that the compounds which co-elute on the DB-5 are partially separated on the SPB-608 and are fully resolved on the DB-210. Both of these capillary columns fully resolved closely eluting peaks pairs, such as TEPP and DDVP, which were not well separated on any of the

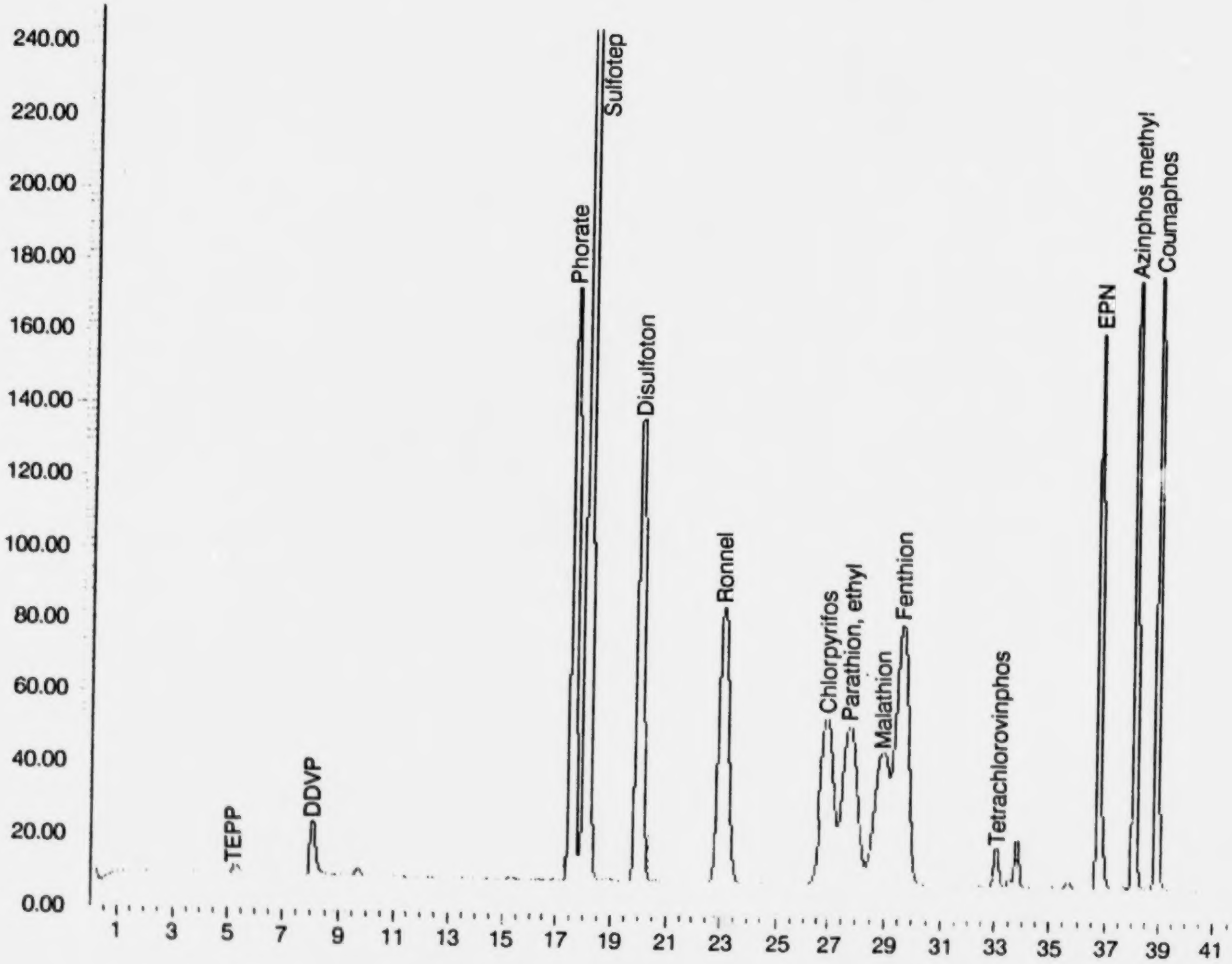


Figure 3. Fourteen organophosphorous insecticides separated on a SPB-608 megabore capillary column.

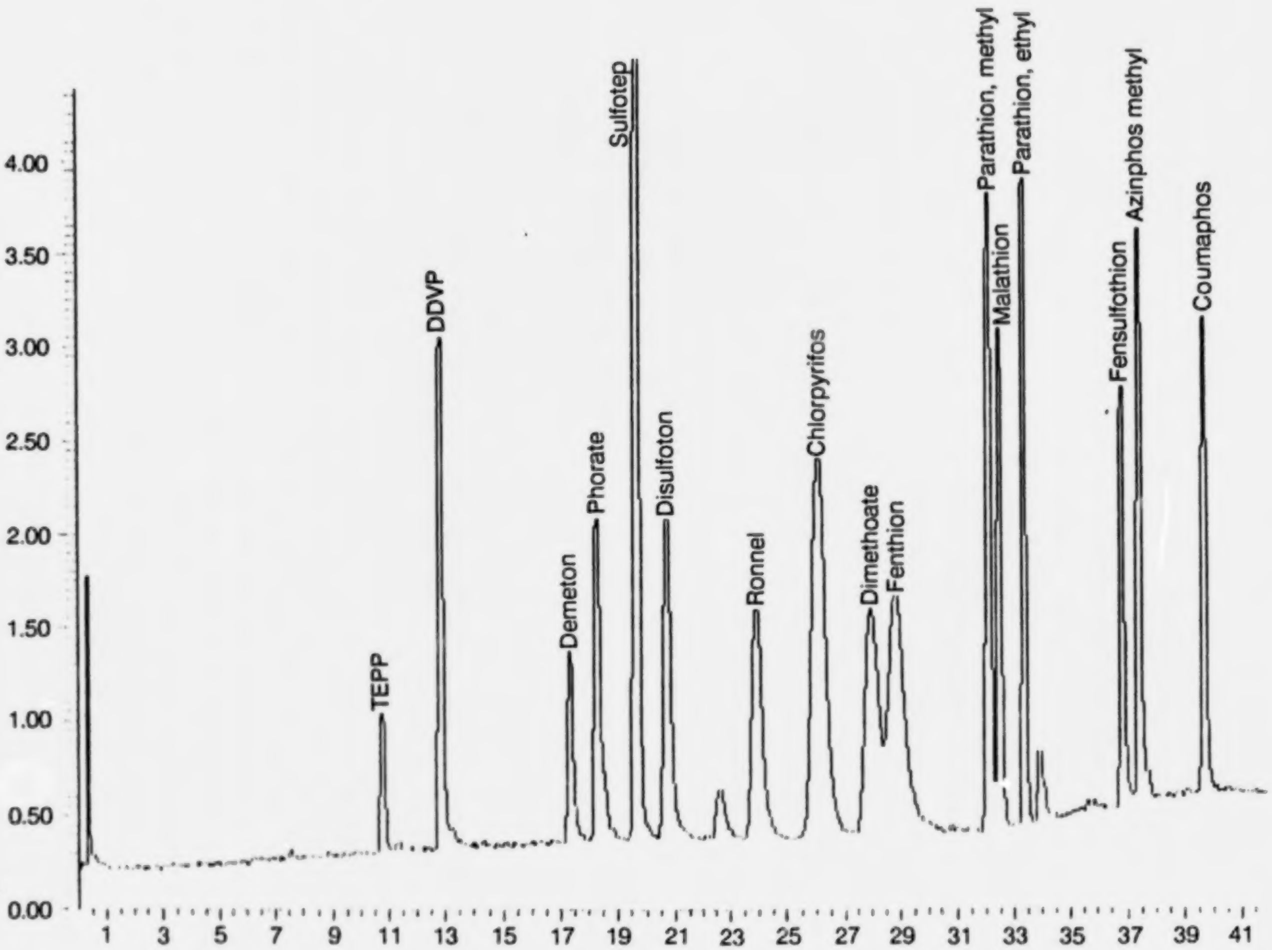


Figure 4. Sixteen organophosphorous insecticides separated on a DB-210 megabore capillary column.

packed columns tested. The retention times for the 27 analytes tested on all three capillary columns are presented in Table 5 along with the optimized temperature programs.

GC Column Recommendations–

Use of a megabore capillary column in place of a packed column provides an improved analysis method for organophosphorous insecticides. The packed columns tested in this study would resolve many of the method parameters, but no column pair could be used to resolve all compounds tested. By using the DB-210 column alone, 21 analytes can be fully resolved; by using the SPB-608 column, at least 16 analytes can be fully resolved. The use of these two megabore columns allowed the analysis of all 27 proposed method parameters. Therefore, dual megabore capillary column analysis is recommended for environmental samples using the DB-210 (or equivalent) and the SPB-608 (or equivalent) columns.

Phosphorous Specific Detector Evaluation

Two phosphorous specific detectors, FPD and NPD, were evaluated as part of this study. The FPD detects by the phosphorous emission of analytes in a hydrogen flame by using a photomultiplier tube and a 526 nm wide-band filter. The NPD is an ionization detector modified by placement of a bead of rubidium doped ceramic on the flame tip. The rubidium alters the flame chemistry to ionize nitrogen and phosphorus species preferentially.[14]

Detector Evaluation Method–

Multicomponent organophosphorous standards were prepared in hexane and single injections were made at five concentrations over a range of 0.5 to 500 μg/ml on either DB-210 or SPB-608 columns using both the NPD and FPD detectors. Linearity and sensitivity for naled and trichloronate were determined on the FPD only. A total of 27 compounds were combined in two mixtures which were selected so that the analytes were completely resolved during GC analysis in order to insure accurate quantitation. Linearity plots of peak area versus concentration were prepared and presented in Figures 5 to 31. As the linearity data was taken from two different data systems, the curves may be compared directly while the magnitude of the response presented on the individual graphs may not be compared directly.

Sample chromatograms for both detectors are presented in Figures 32 (FPD) and 33 (NPD). The nitrogen-phosphorous detector exhibited considerable peak broadening which was probably caused by the use of a packed column detector jet with the megabore column. Use of the NPD with megabore requires a megabore specific jet in the detector. Peak broadening was not observed with the FPD using the packed column detector base.

TABLE 5. CAPILLARY COLUMN RETENTION TIMES FOR METHOD 8140 ANALYTES

Compound	Capillary Column	
	SPB608	DB210
Azinphos methyl	38.04	37.24
Bolstar	35.08	37.55
Chlorpyrifos	26.88	25.18
Coumaphos	38.87	39.47
Demeton, O,S	15.90	17.24
Dimethoate	20.18	27.96
Diazinon	20.02	19.68
Dichlorvos	7.91	12.79
Disulfoton	19.96	20.66
Ethoprop	16.48	18.67
EPN	36.71	36.74
Fensulfothion	35.20	36.80
Fenthion	29.45	28.86
Malathion	28.78	32.58
Merphos	21.73	32.44
Mervinphos	12.88	18.44
Monocrotrophos	20.11	31.42
Naled	17.40	19.35
Parathion, ethyl	27.62	33.39
Parathion, methyl	23.71	32.17
Phorate	17.52	18.19
Ronnel	22.98	23.19
Sulfotep	18.02	19.58
TEPP	5.12	10.66
Tetrachlorovinphos	32.99	33.68
Tokuthion	24.58	39.94
Trichloronate	28.41	29.95

Temperature Program used for Both Capillary Columns

Initial temperature	50°C
Initial time	1 minute
Rate	5°C/minute
Final temperature	140°C
Hold 1	10 minutes
Rate	10°C/minute
Final temperature	240°C
Hold 2	10 minutes

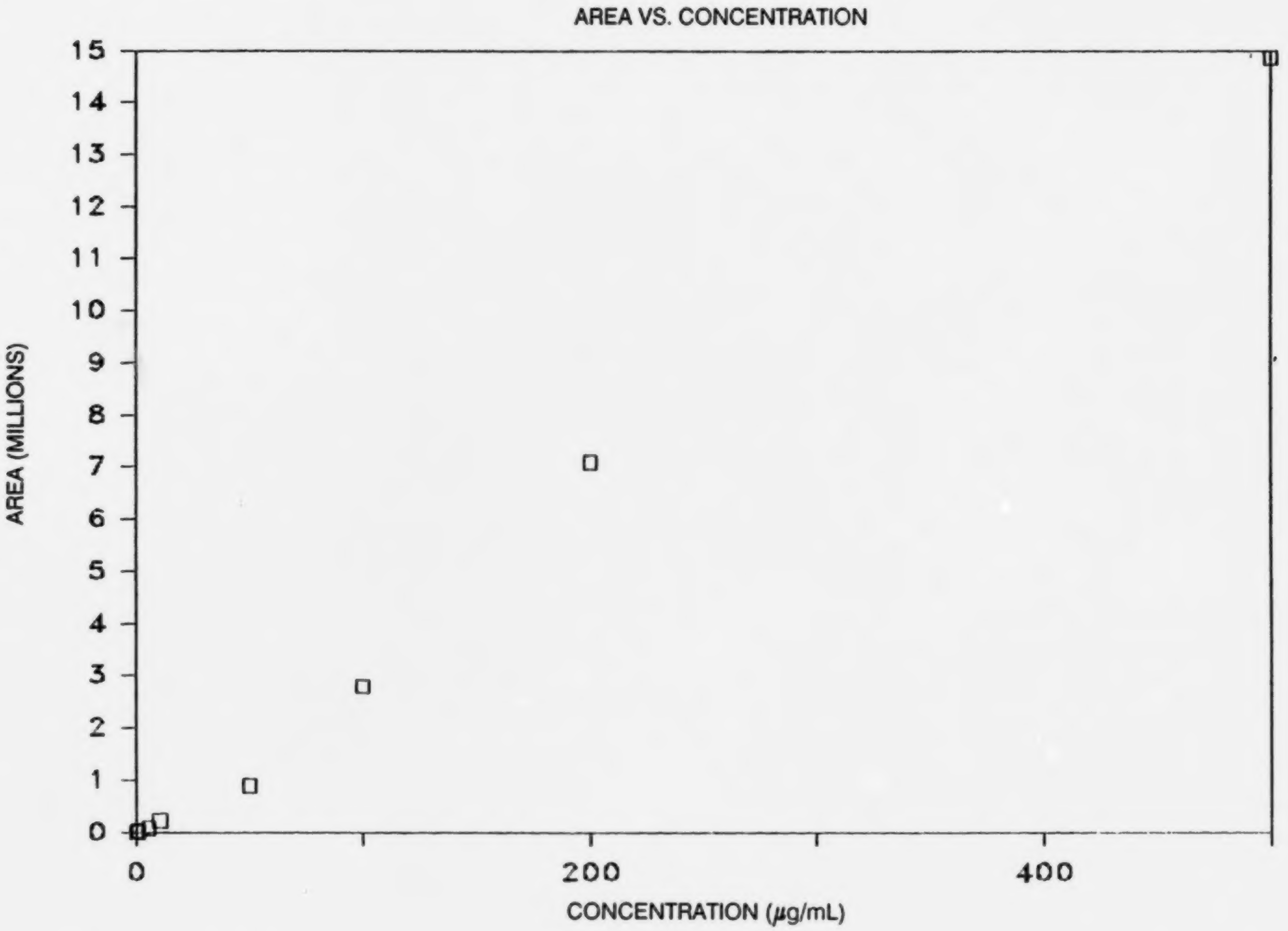


Figure 5A. Azinphos methyl: Response (arbitrary units) vs. concentration on the NPD detector.

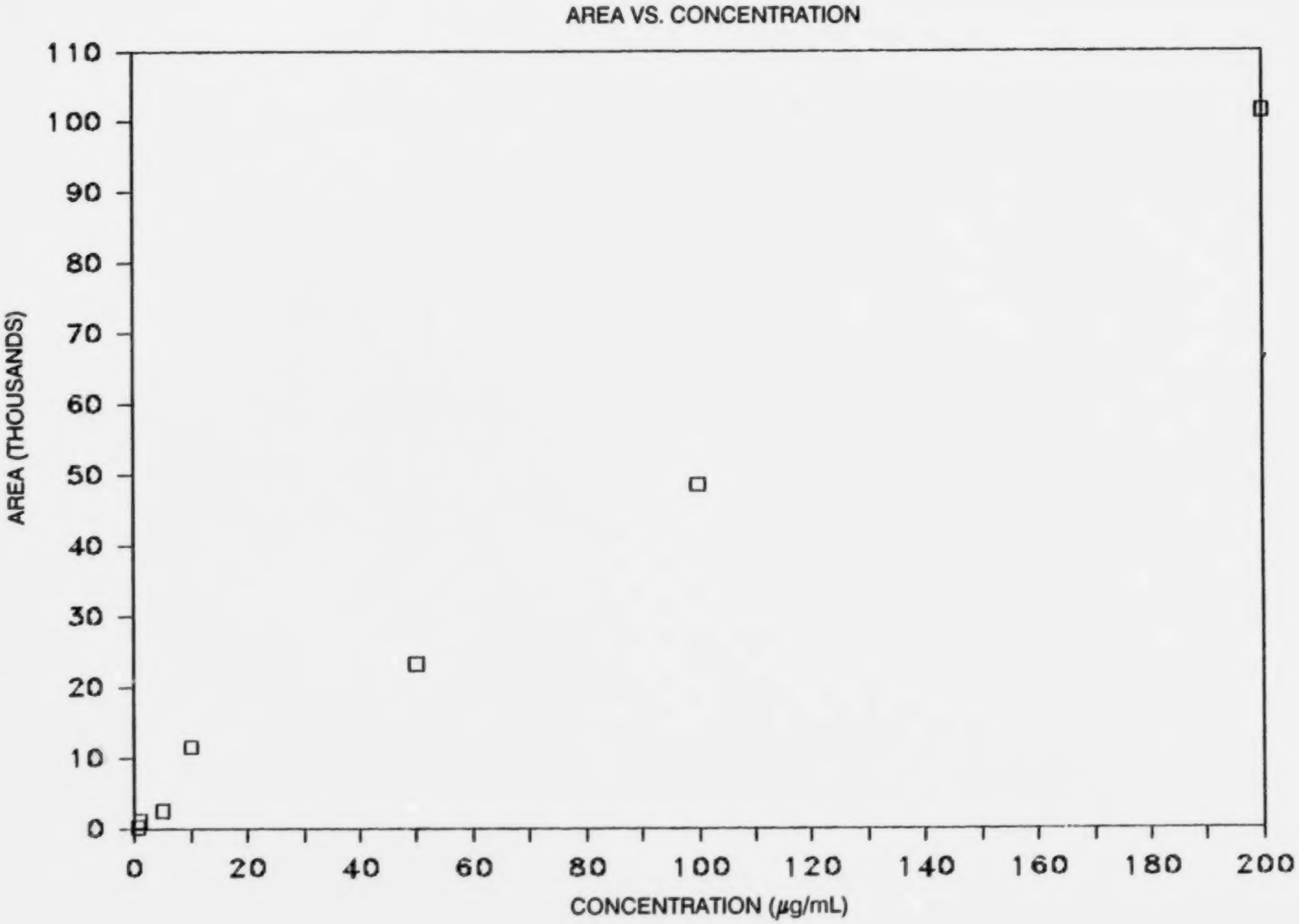


Figure 5B. Azinphos methyl: Response (arbitrary units) vs. concentration on the FPD detector.

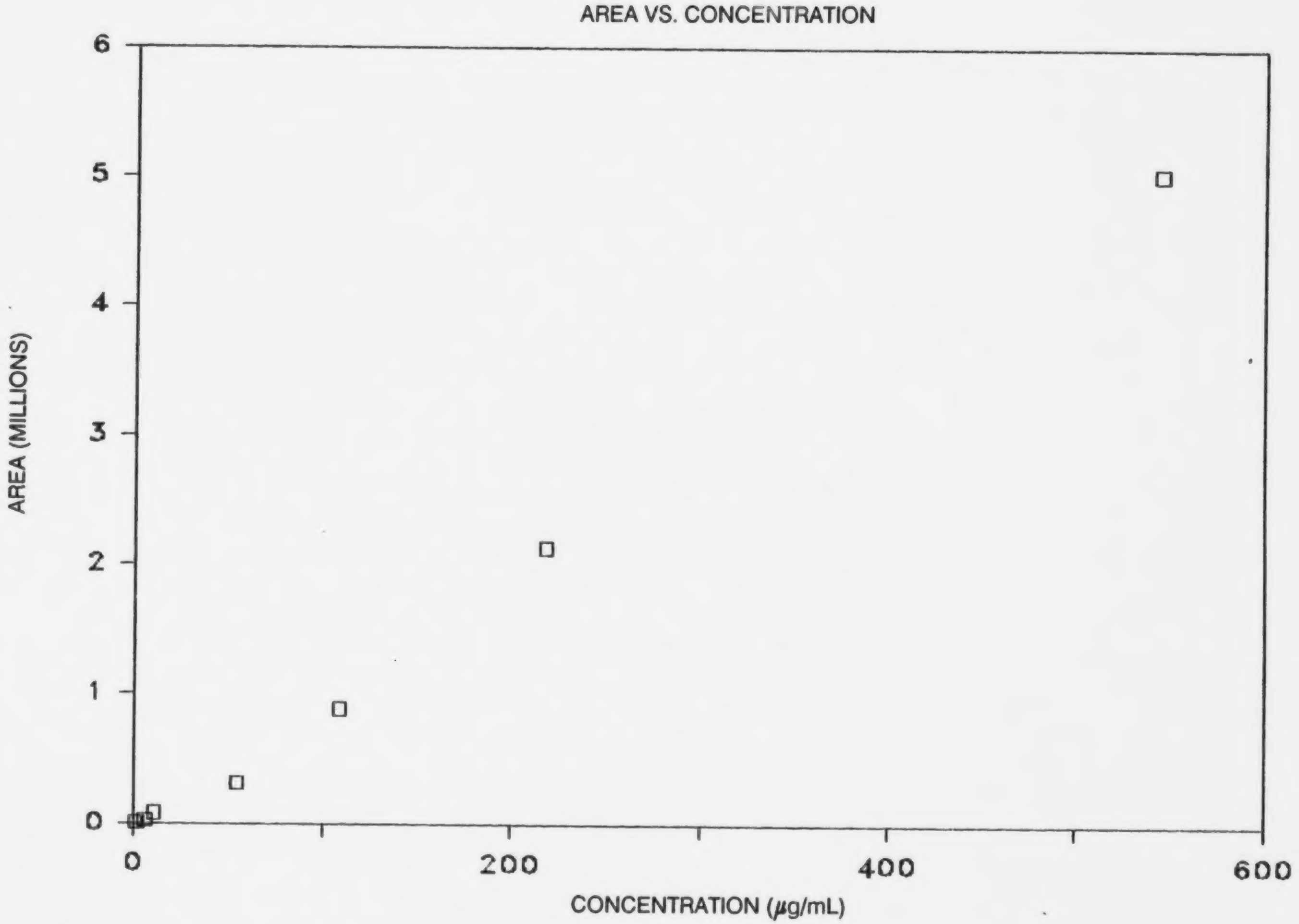


Figure 6A. Bolstar: Response (arbitrary units) vs. concentration on the NPD detector.

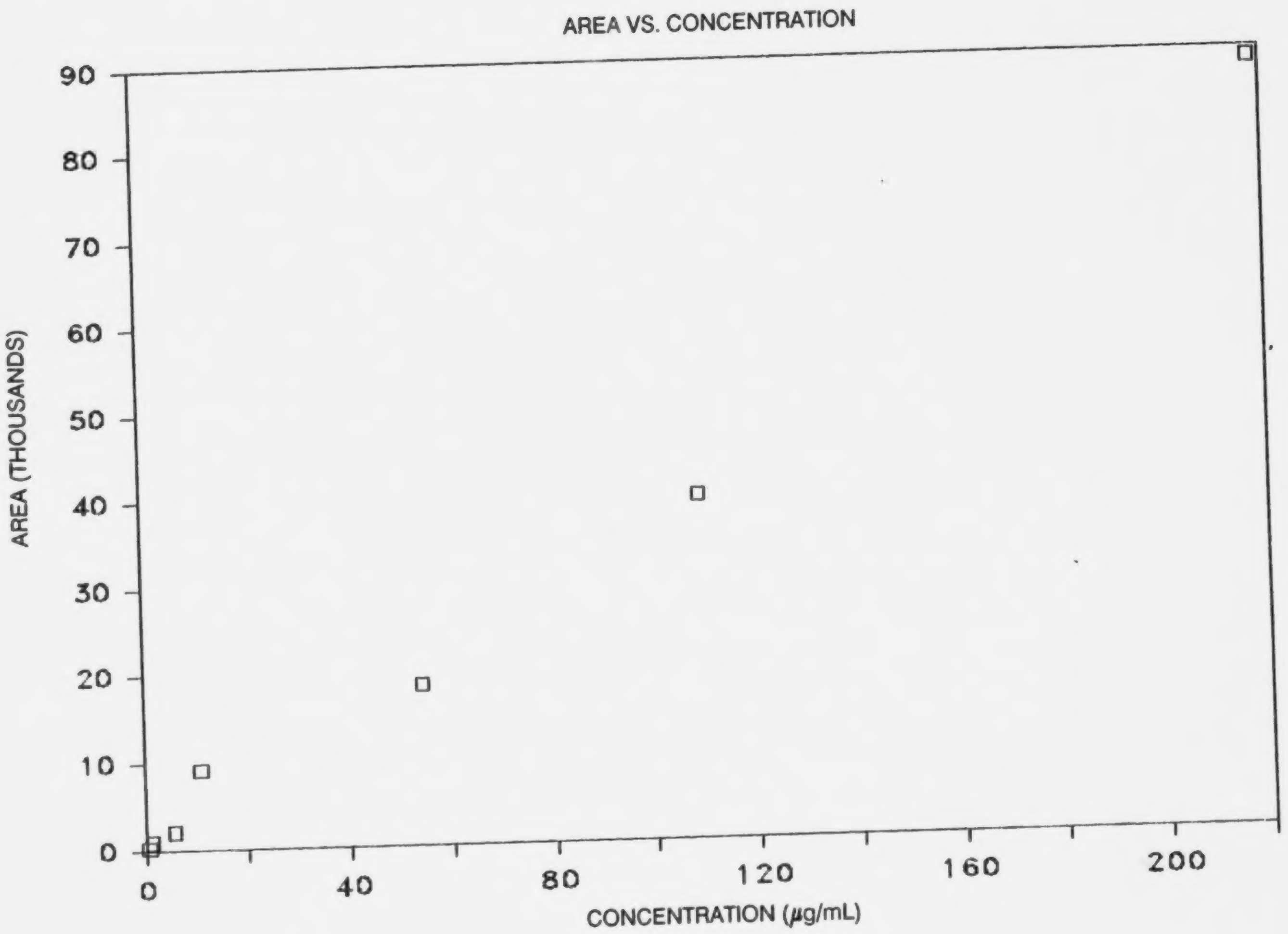


Figure 6B. Bolstar: Response (arbitrary units) vs. concentration on the FPD detector.

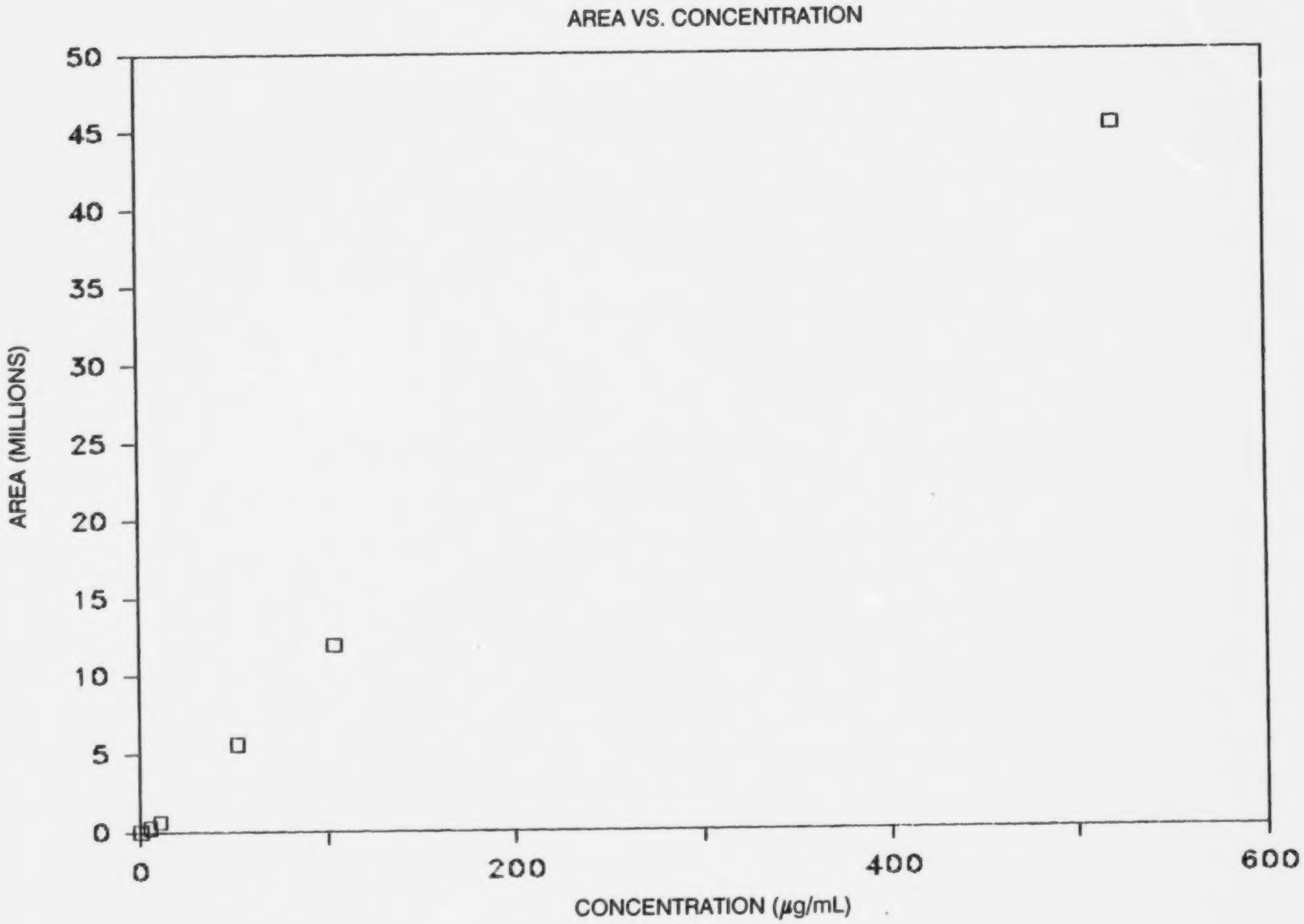


Figure 7A. Chlorpyrifos: Response (arbitrary units) vs. concentration on the NPD detector.

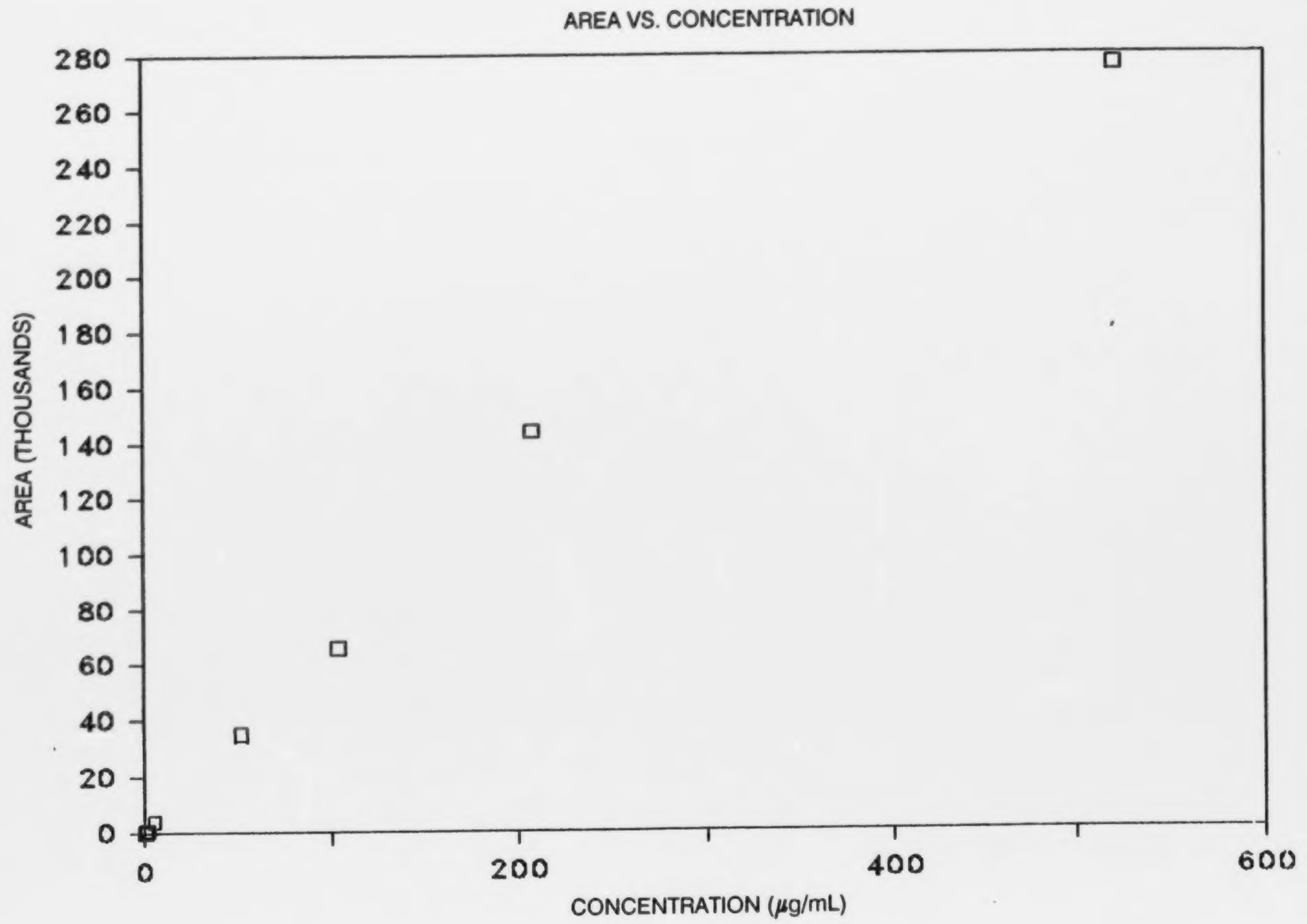


Figure 7B. Chlorpyrifos: Response (arbitrary units) vs. concentration on the FPD detector.

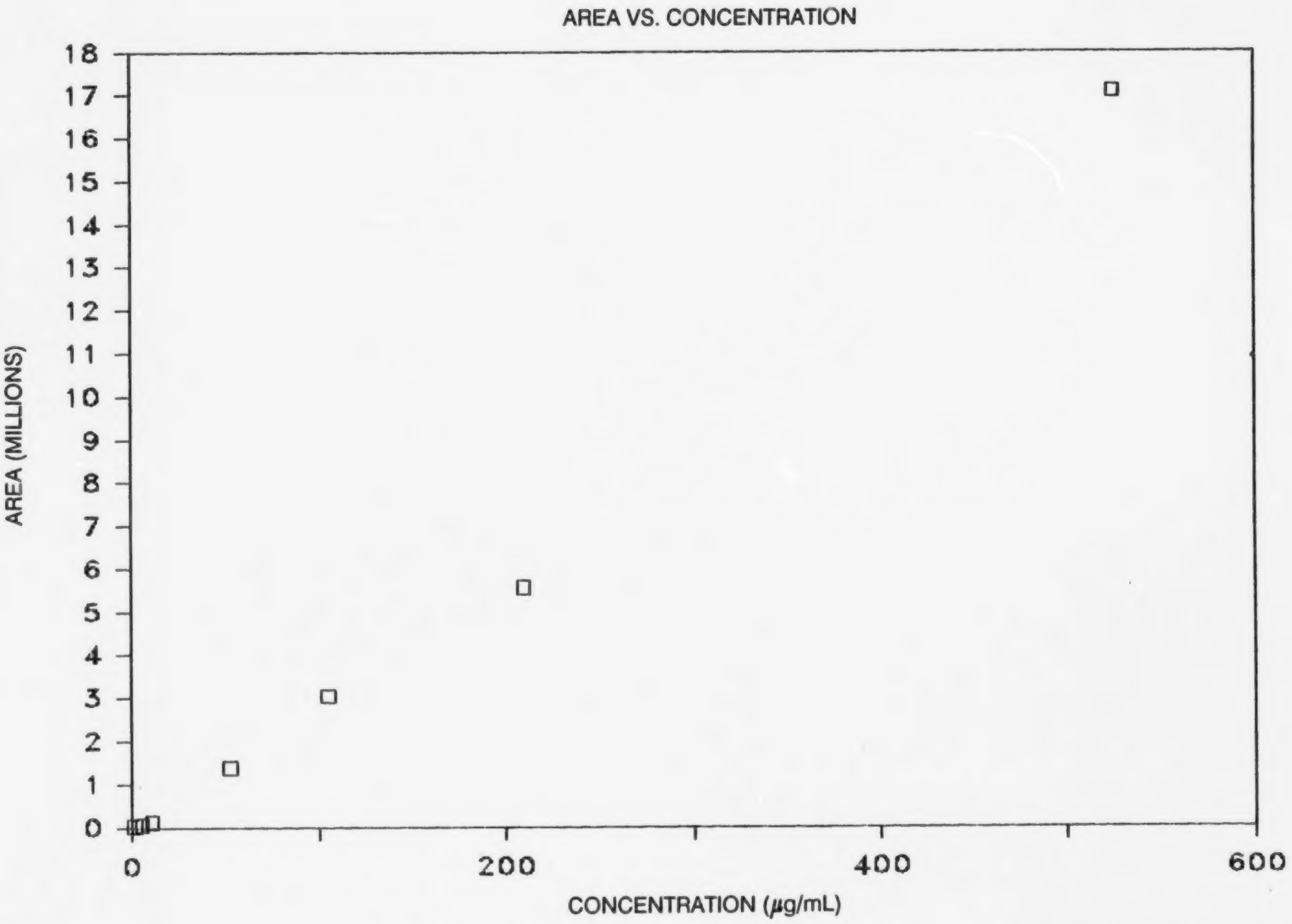


Figure 8A.   Coumaphos: Response (arbitrary units) vs. concentration on the NPD detector.

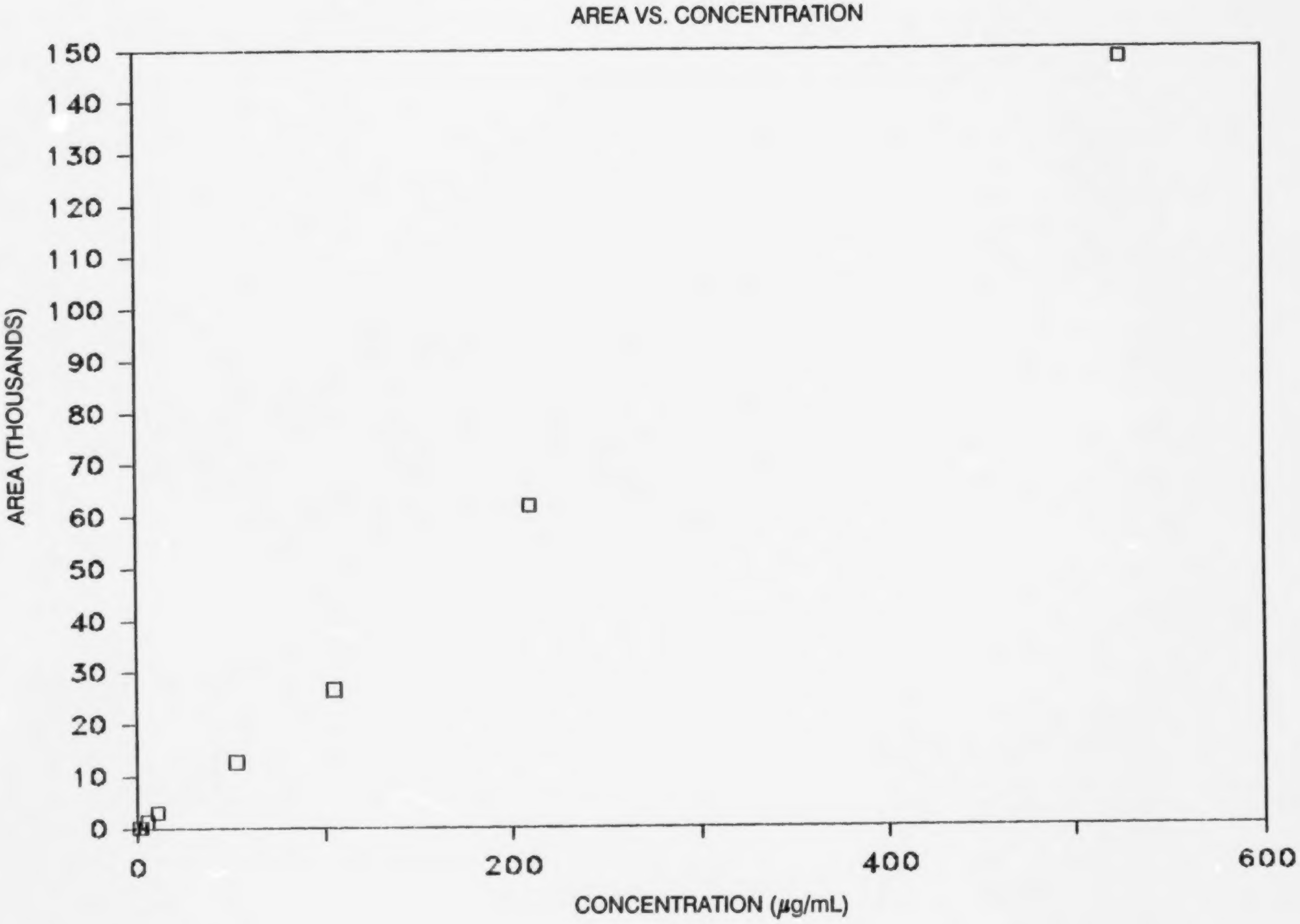


Figure 8B. Coumaphos: Response (arbitrary units) vs. concentration on the FPD detector.

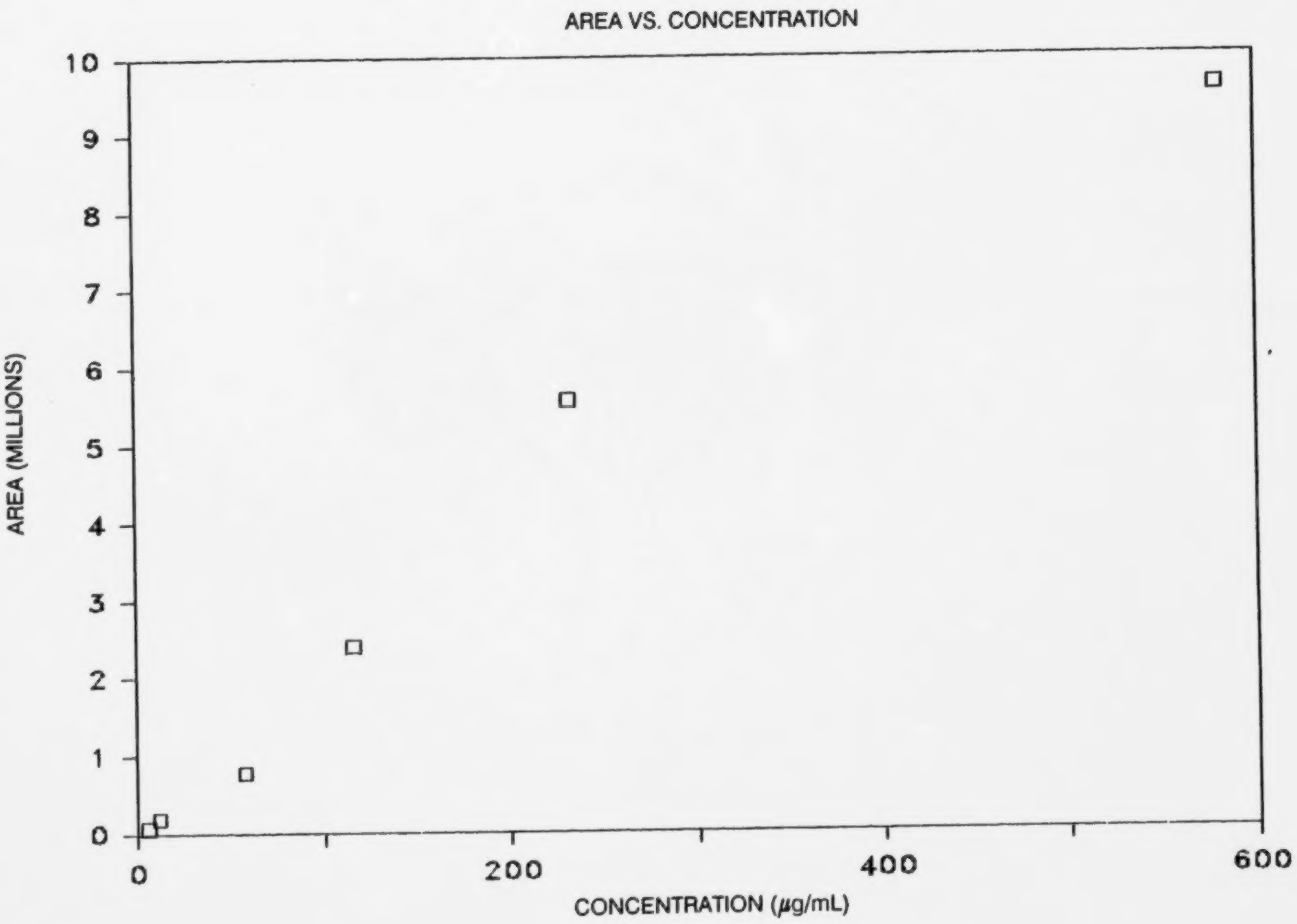


Figure 9A. Diazinon: Response (arbitrary units) vs. concentration on the NPD detector.

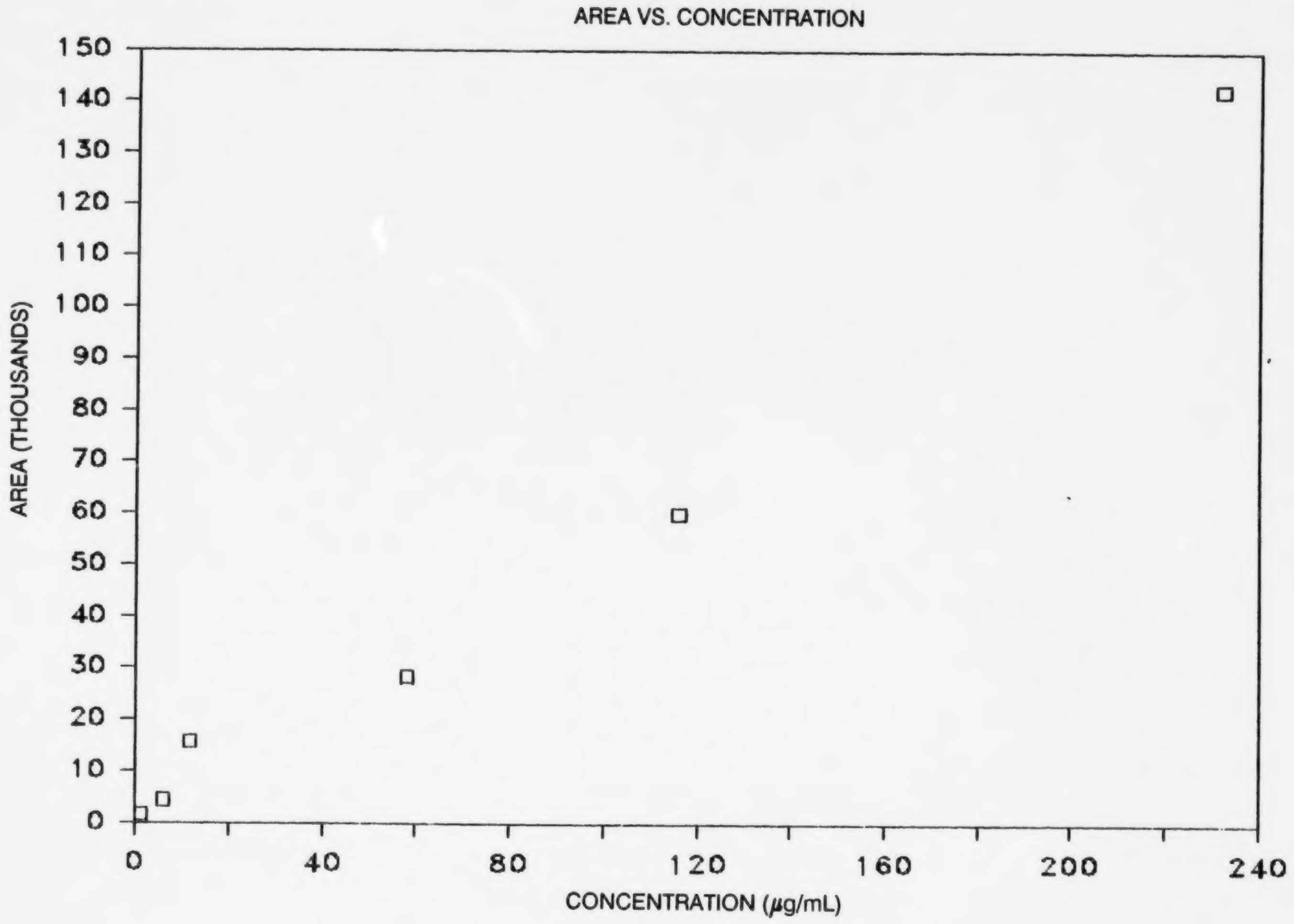


Figure 9B. Diazinon: Response (arbitrary units) vs. concentration on the FPD detector.

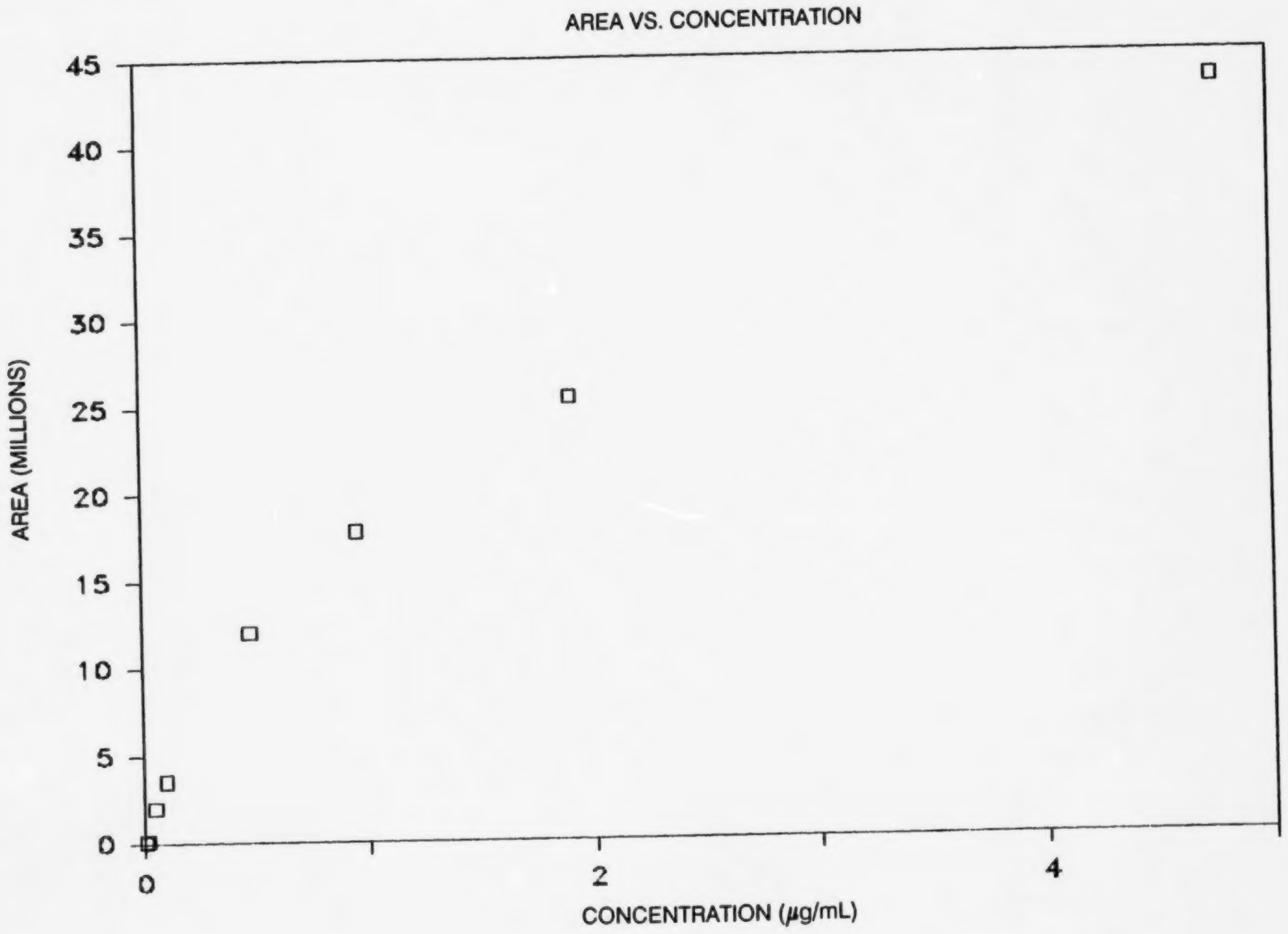


Figure 10A. DDVP: Response (arbitrary units) vs. concentration on the NPD detector.

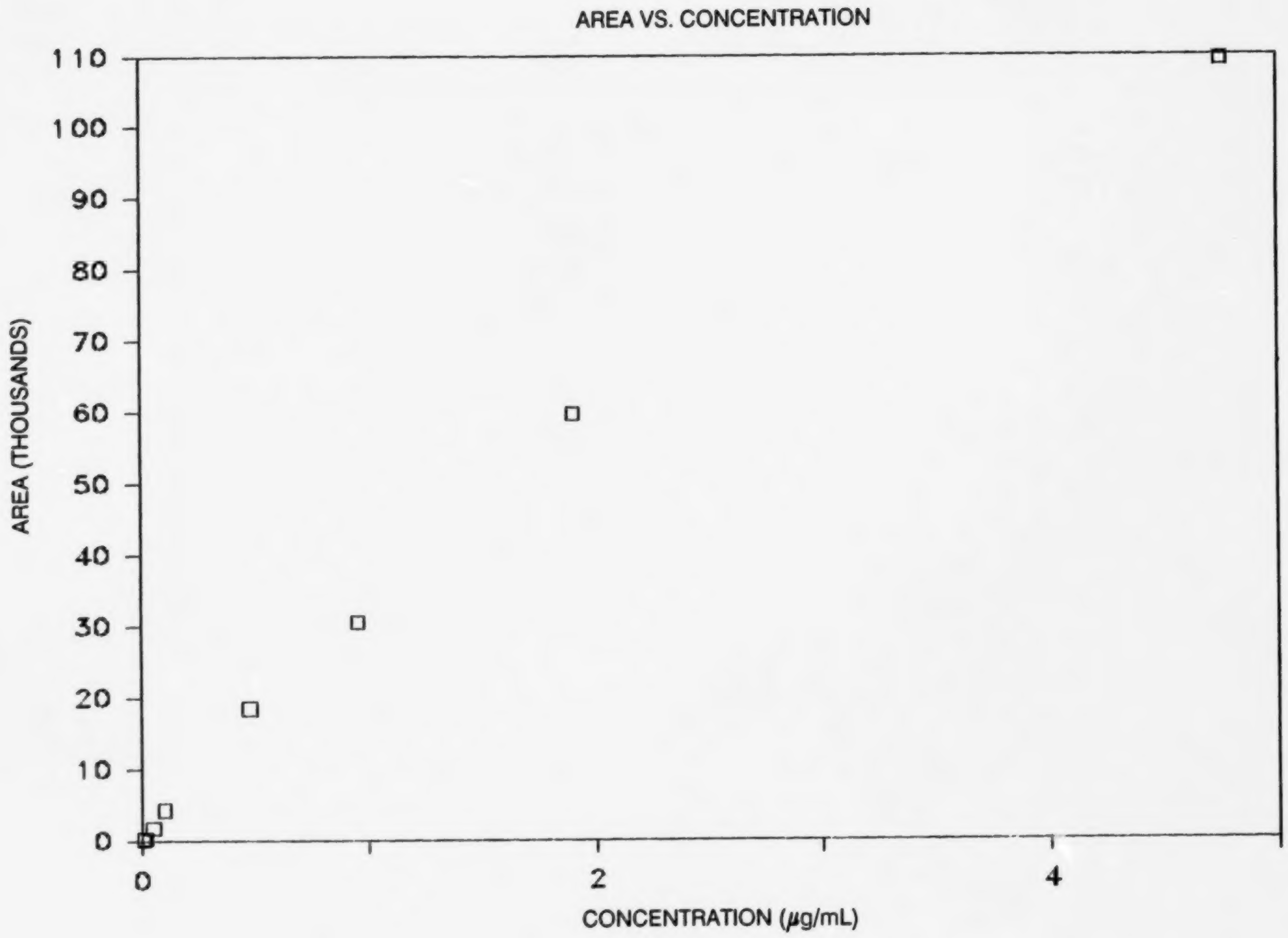


Figure 10B. DDVP: Response (arbitrary units) vs. concentration on the FPD detector.

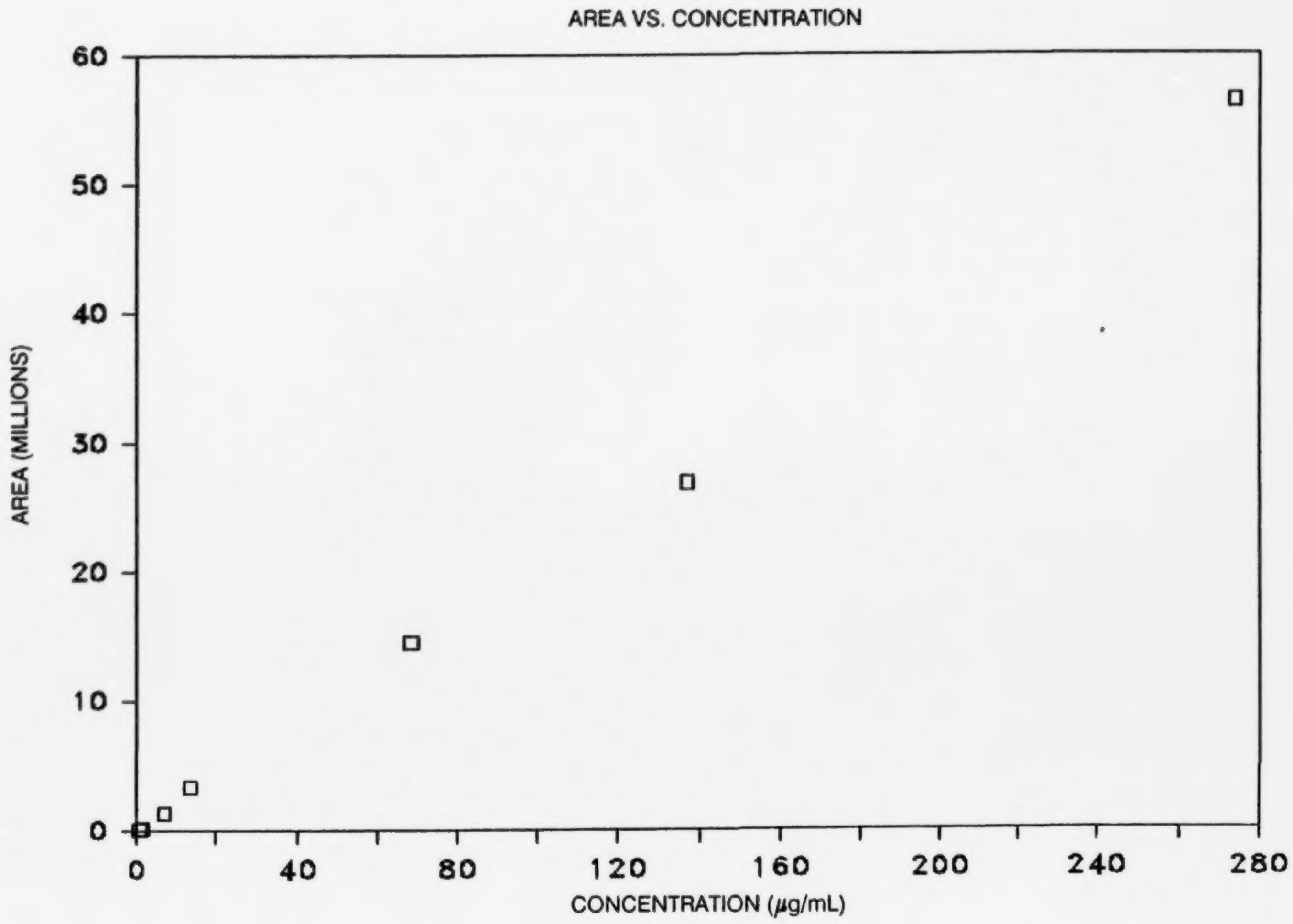


Figure 11A. Demeton: Response (arbitrary units) vs. concentration on the NPD detector.

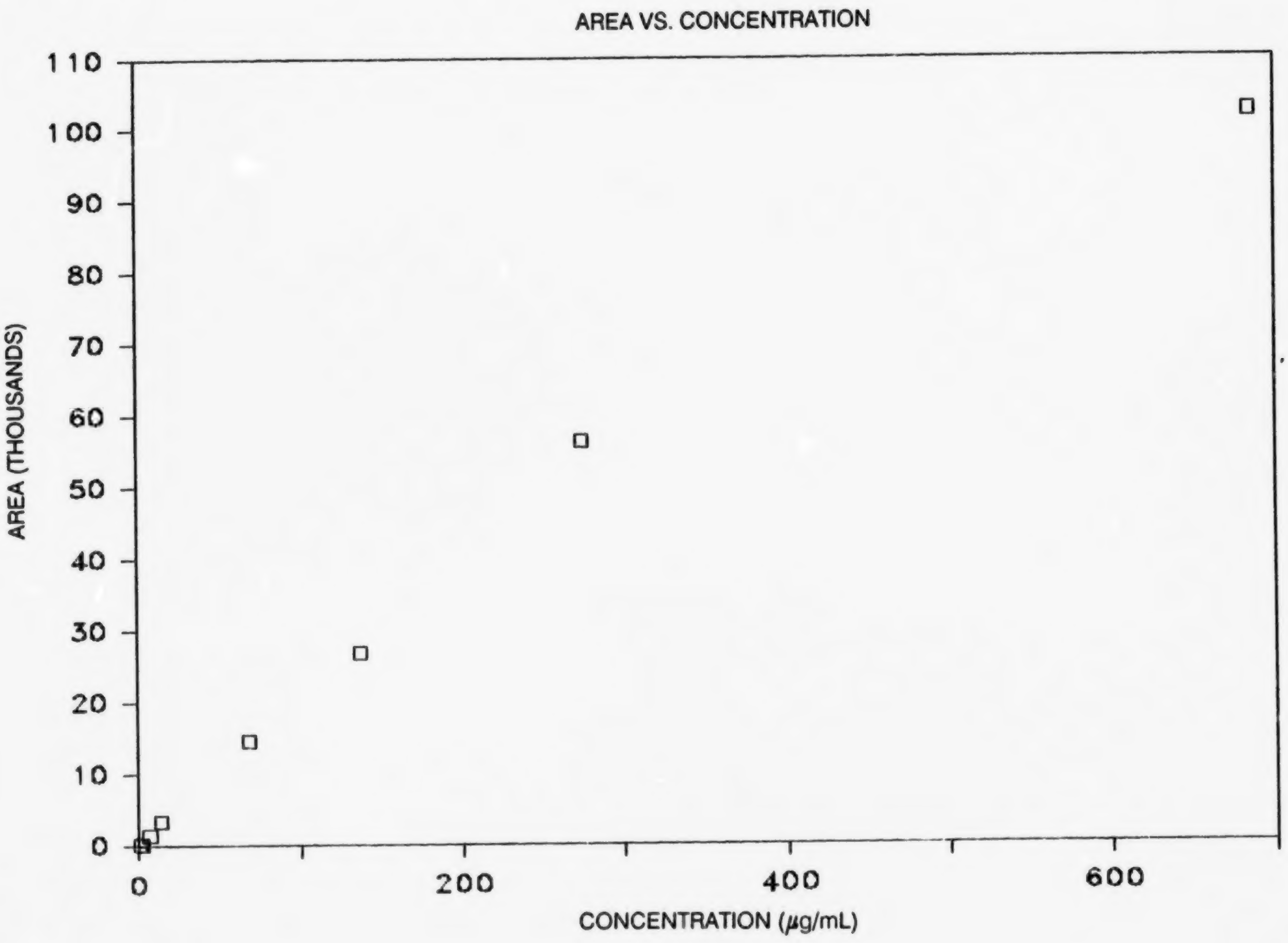


Figure 11B. Demeton: Response (arbitrary units) vs. concentration on the FPD detector.

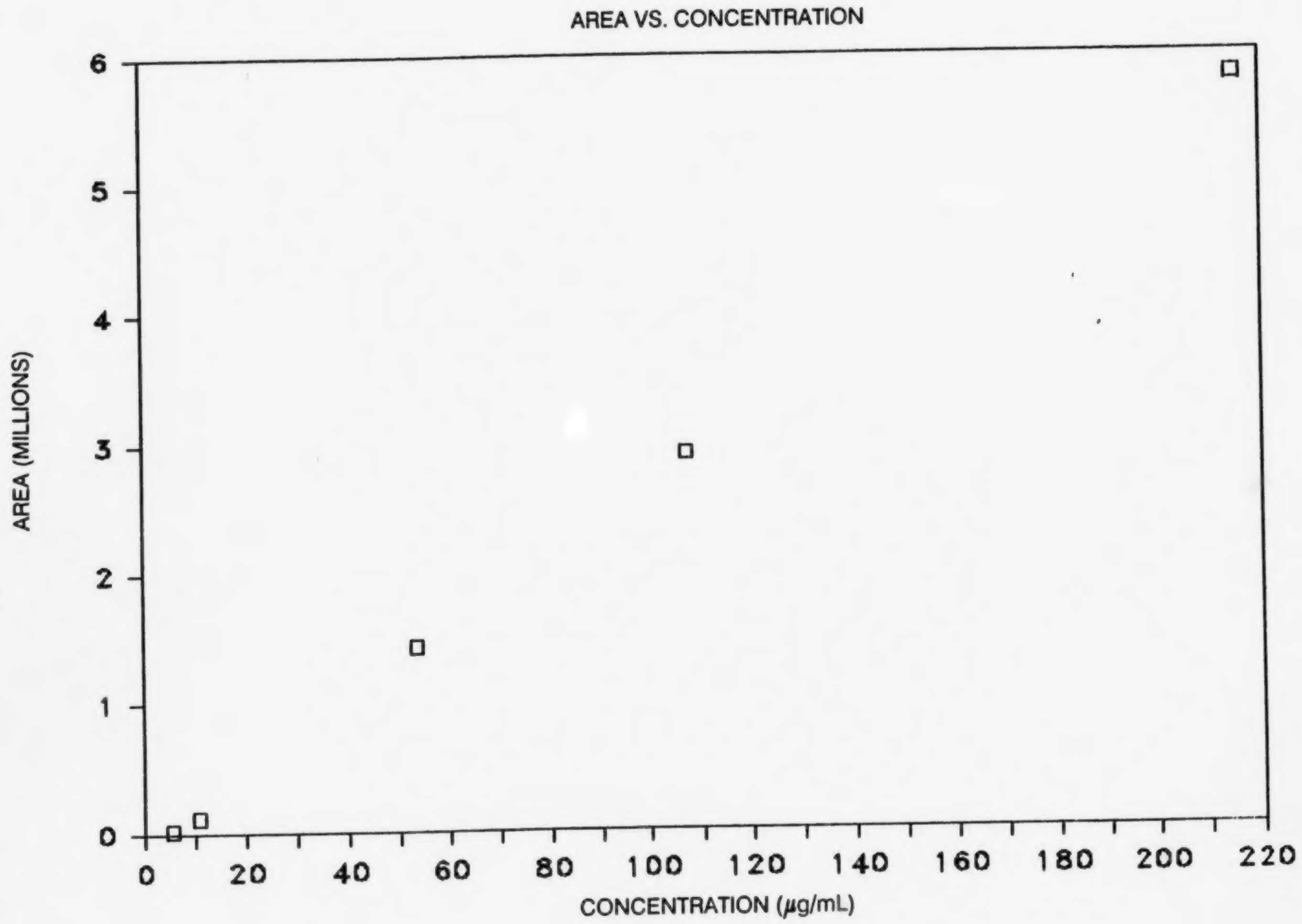


Figure 12A. **Dimethoate: Response (arbitrary units) vs. concentration on the NPD detector.**

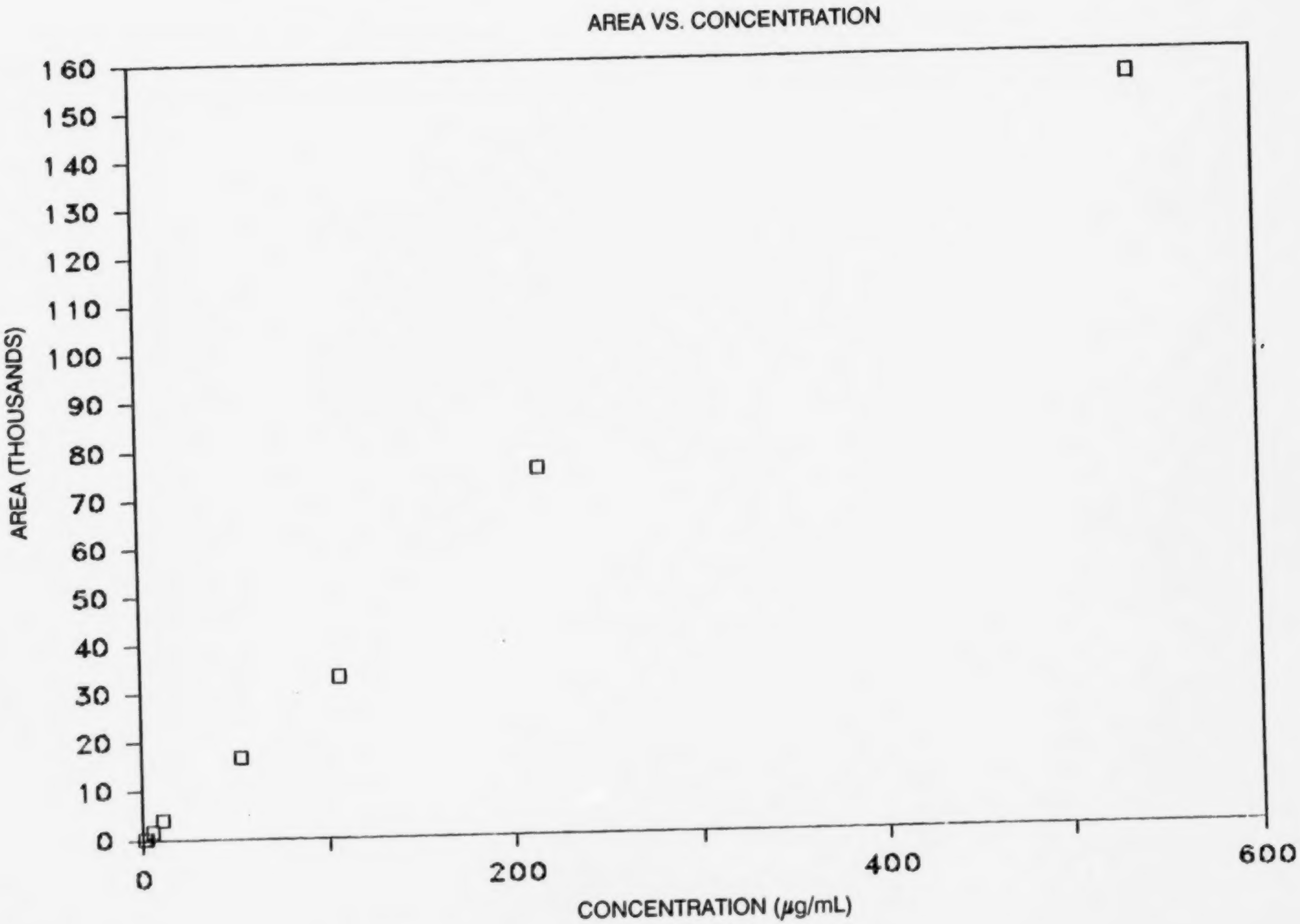


Figure 12B. Dimethoate: Response (arbitrary units) vs. concentration on the FPD detector.

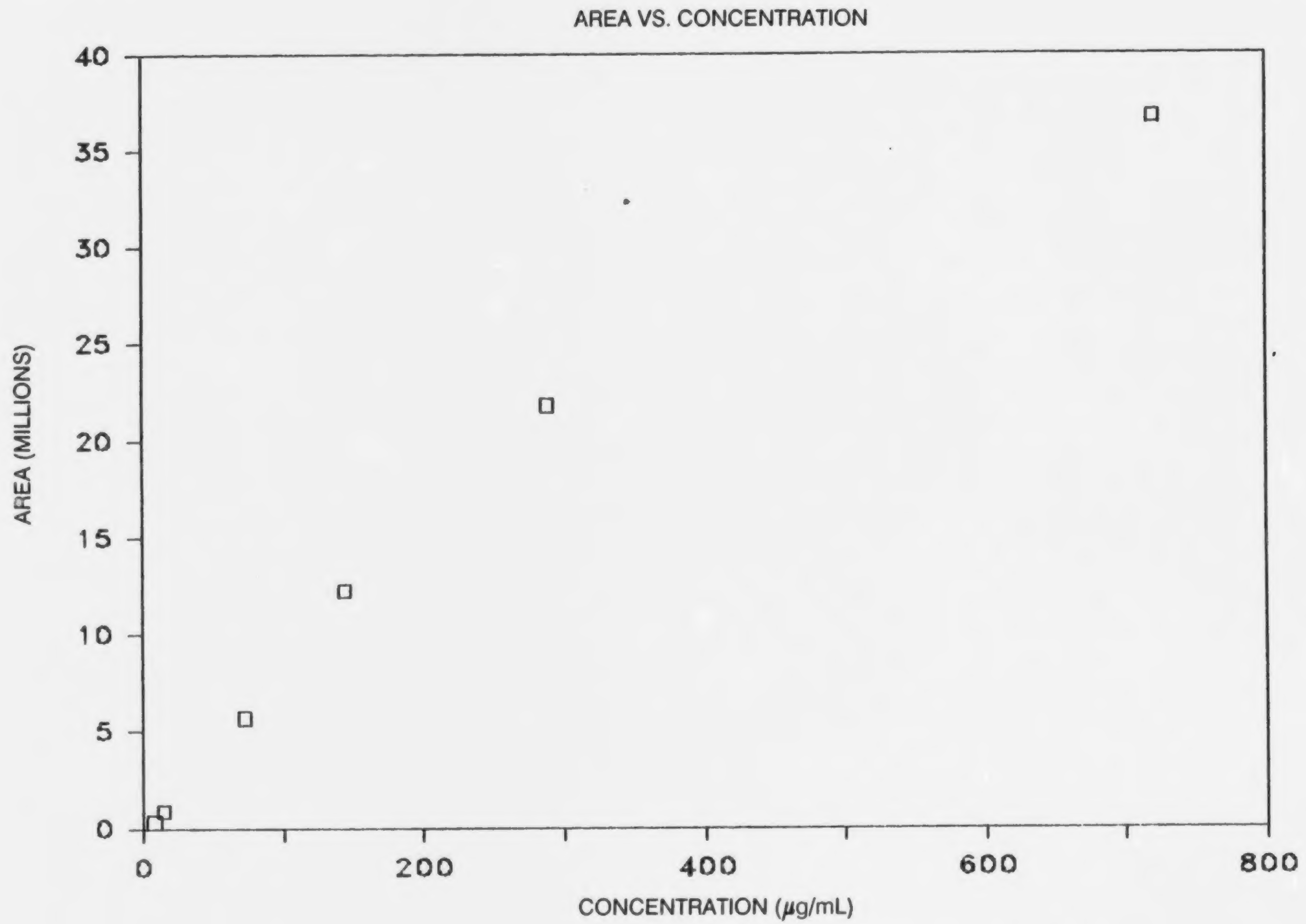


Figure 13A. Disulfoton: Response (arbitrary units) vs. concentration on the NPD detector.

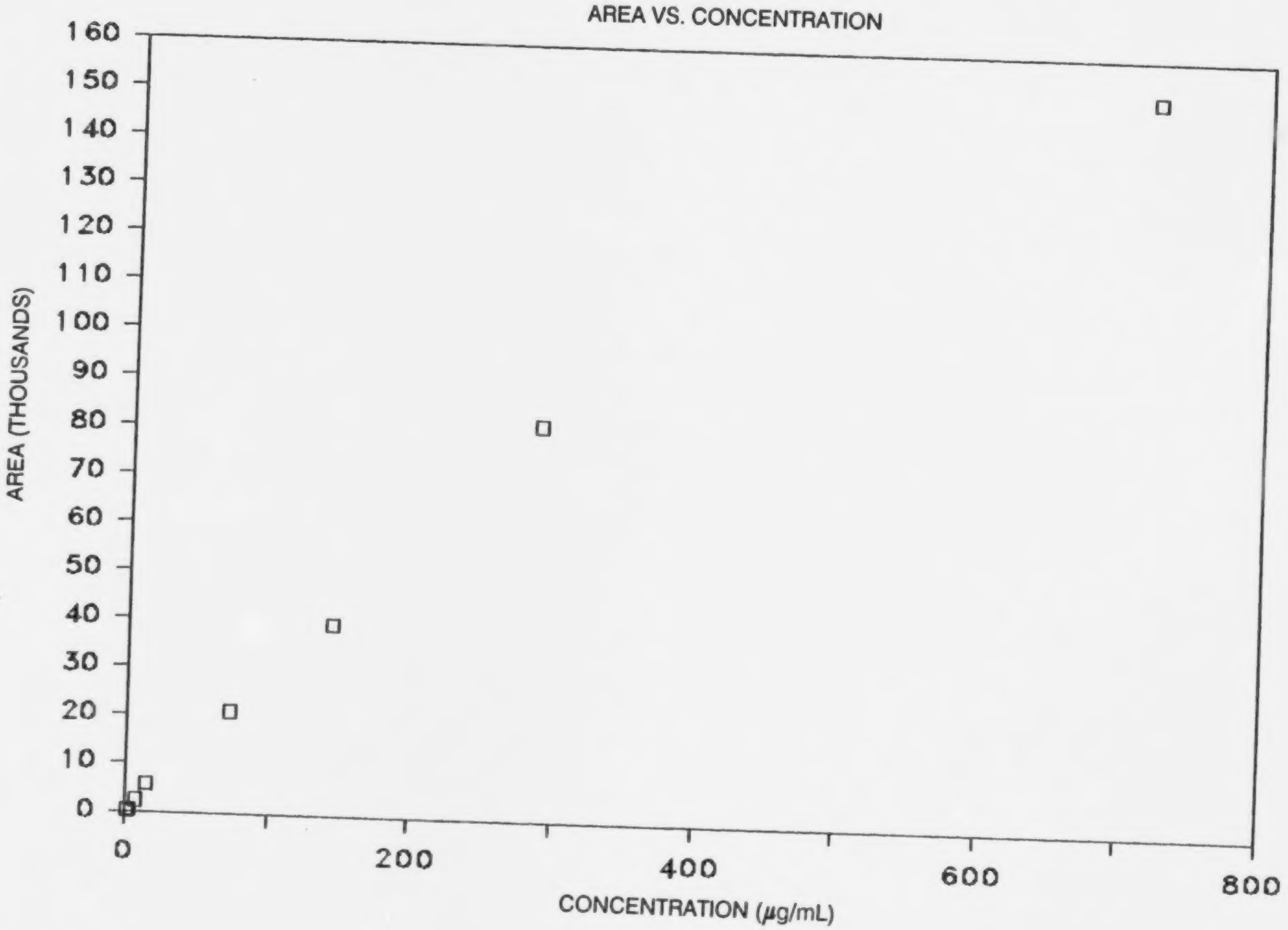


Figure 13B. Disulfoton: Response (arbitrary units) vs. concentration on the FPD detector.

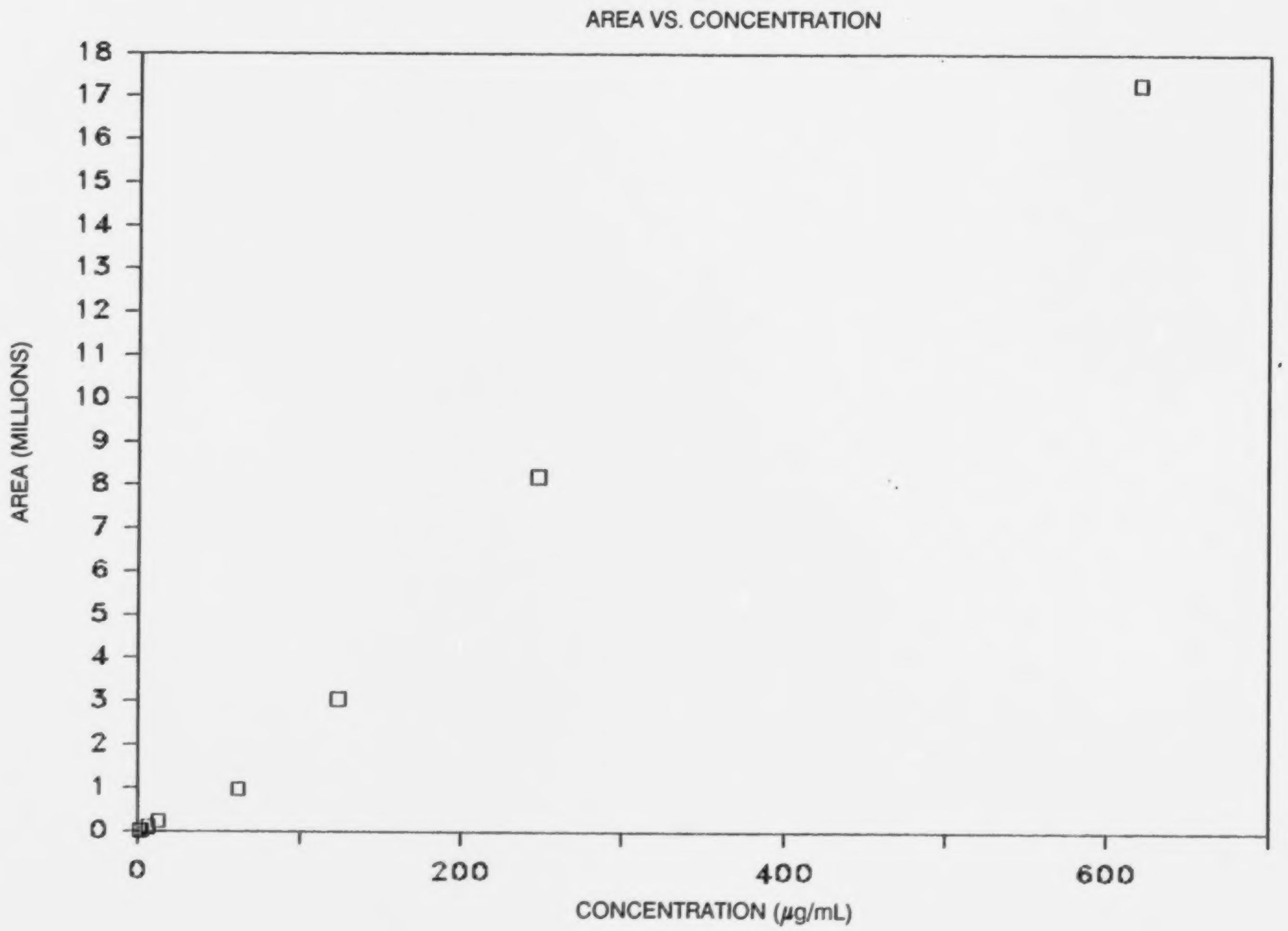


Figure 14A. EPN: Response (arbitrary units) vs. concentration on the NPD detector.

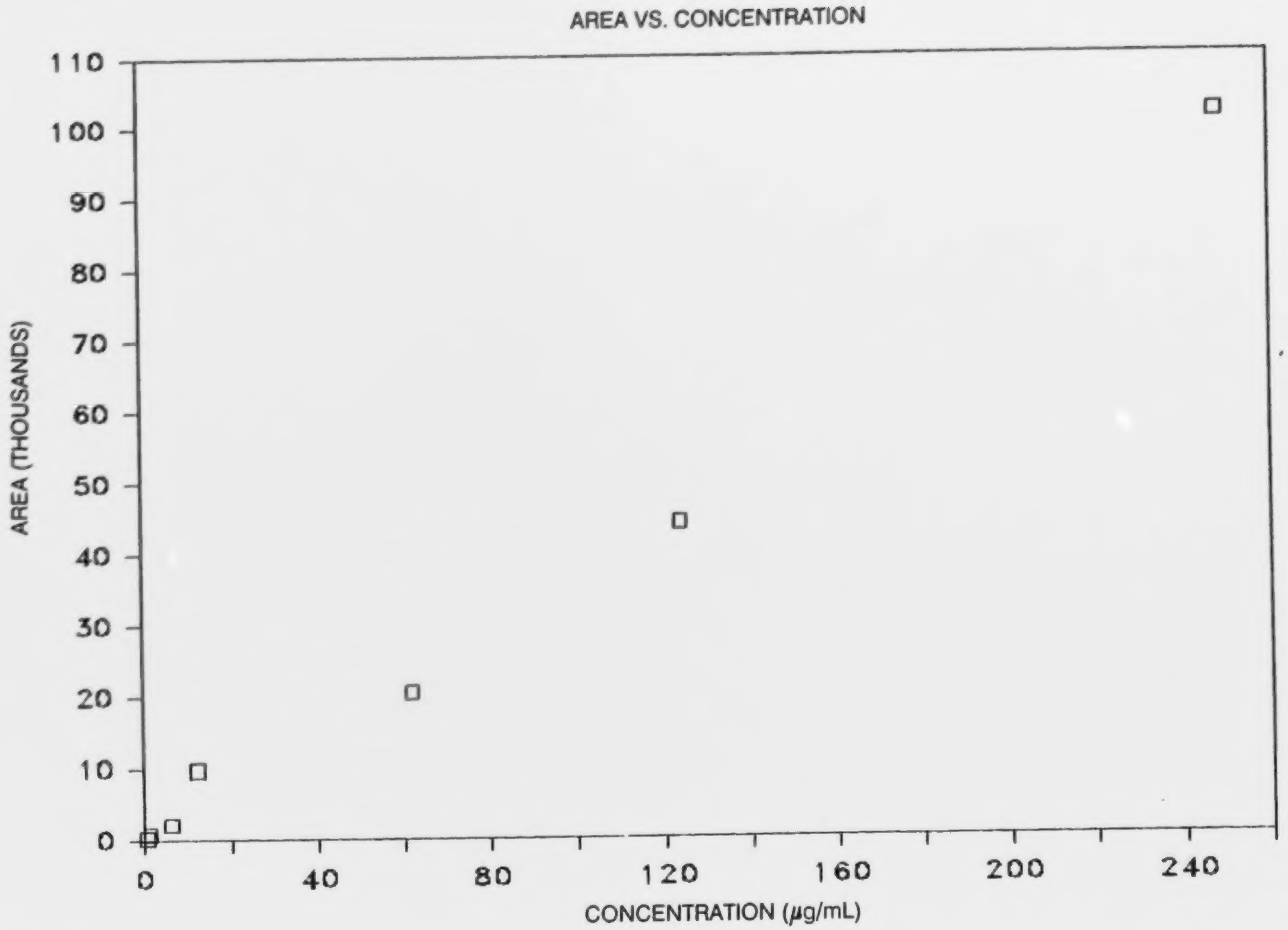


Figure 14B. EPN: Response (arbitrary units) vs. concentration on the FPD detector.

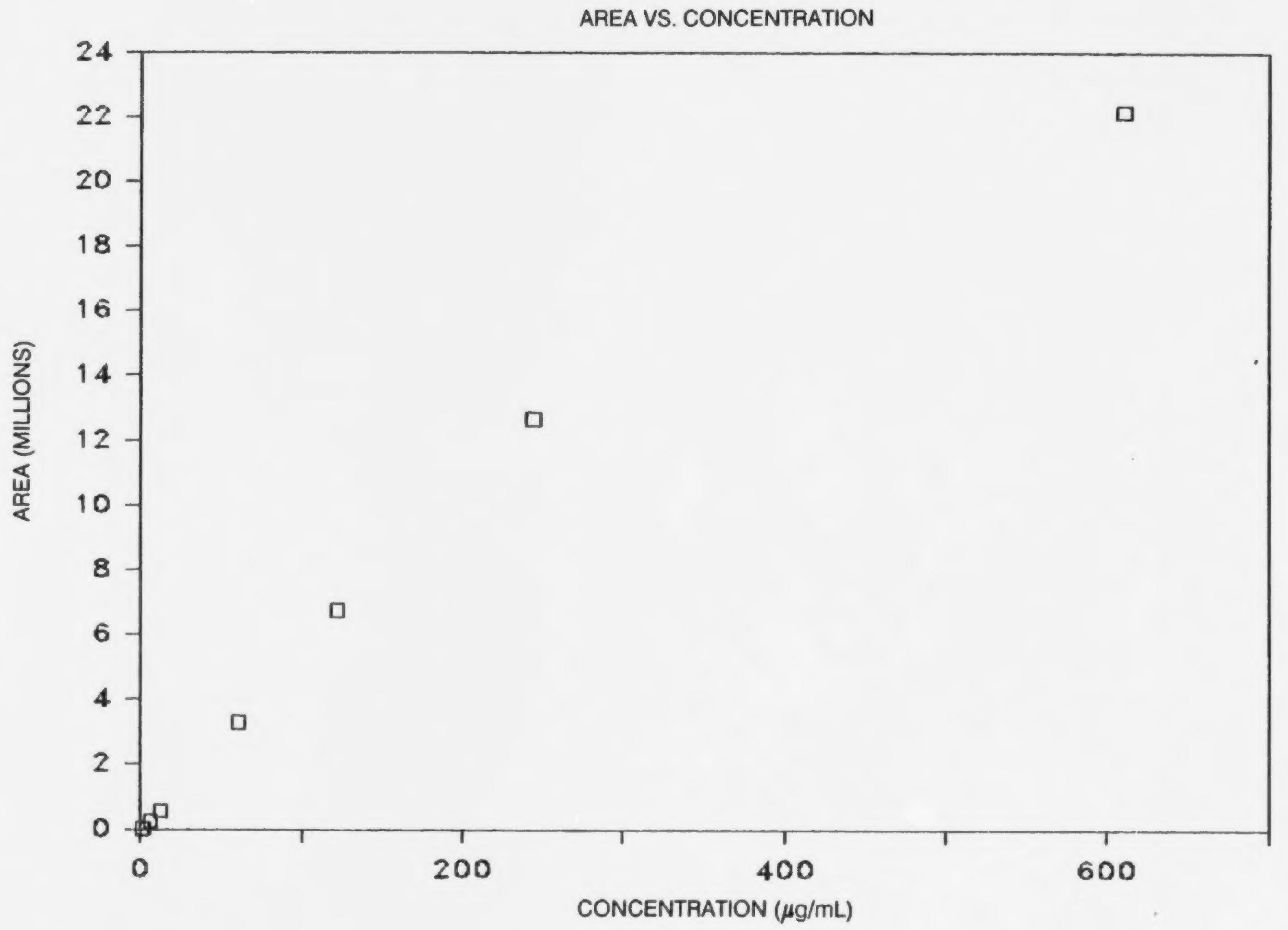


Figure 15A. Ethoprop: Response (arbitrary units) vs. concentration on the NPD detector.

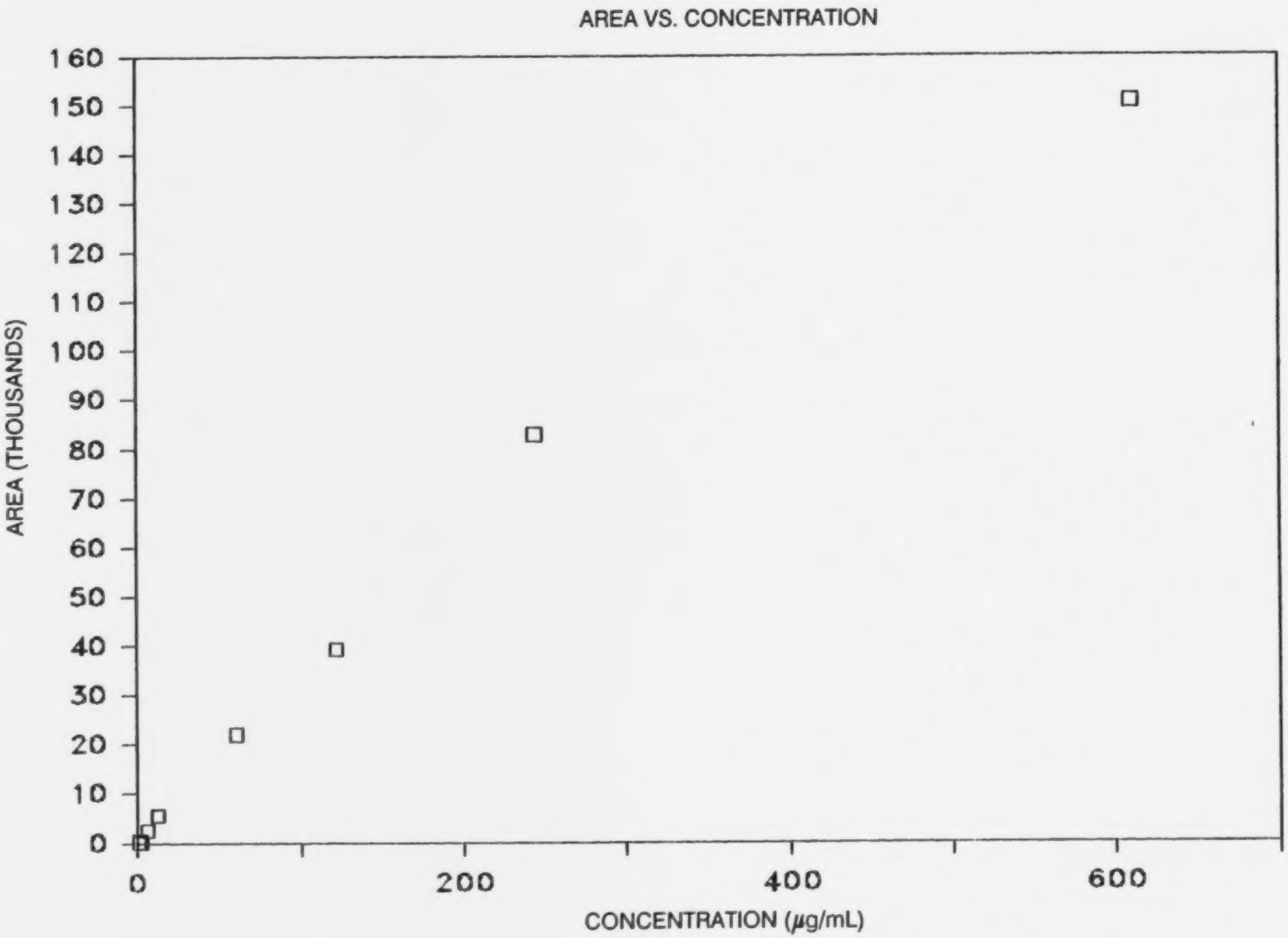


Figure 15B. **Ethoprop: Response (arbitrary units) vs. concentration on the FPD detector.**

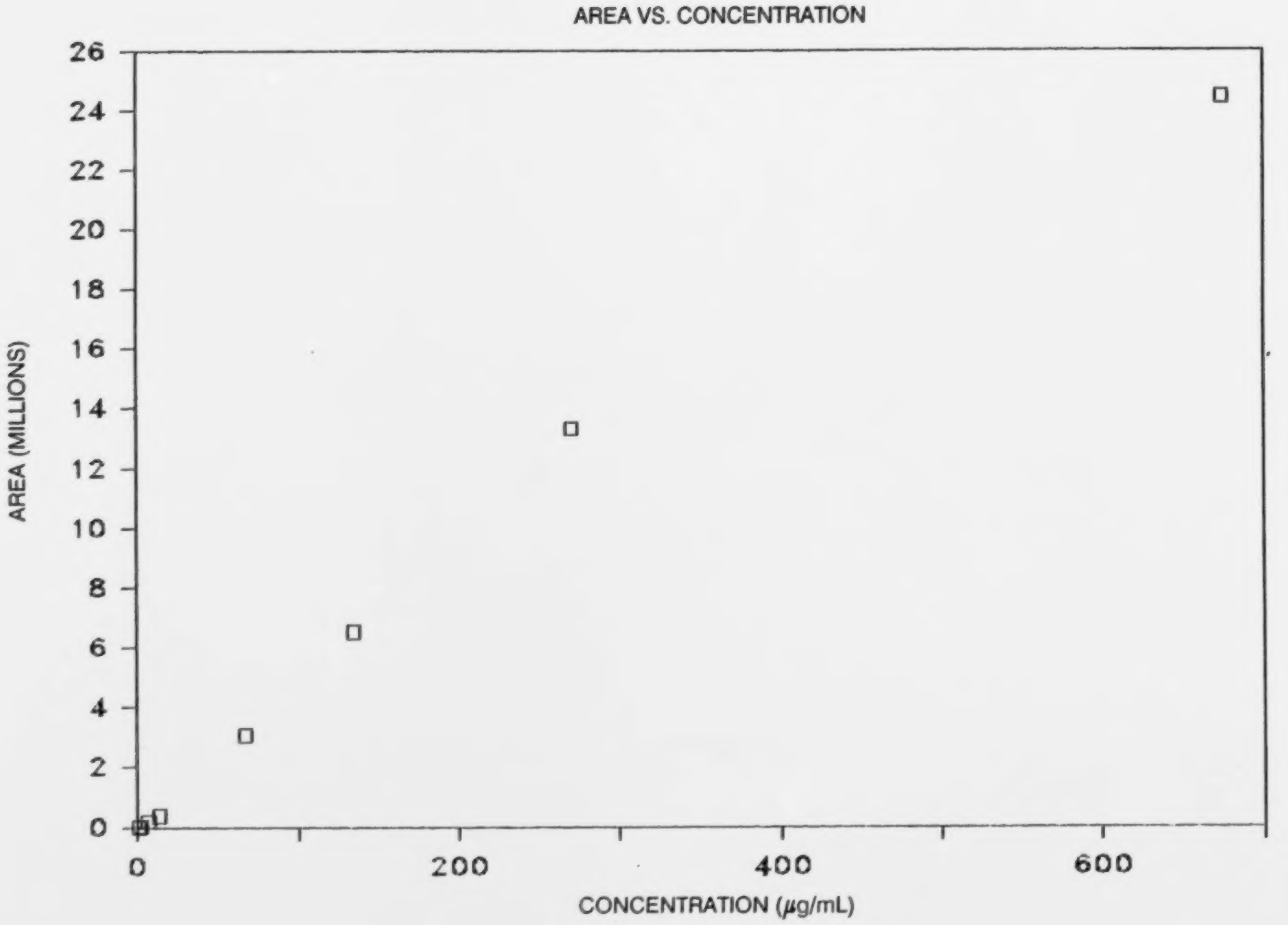


Figure 16A. Ethyl parathion: Response (arbitrary units) vs. concentration on the NPD detector.

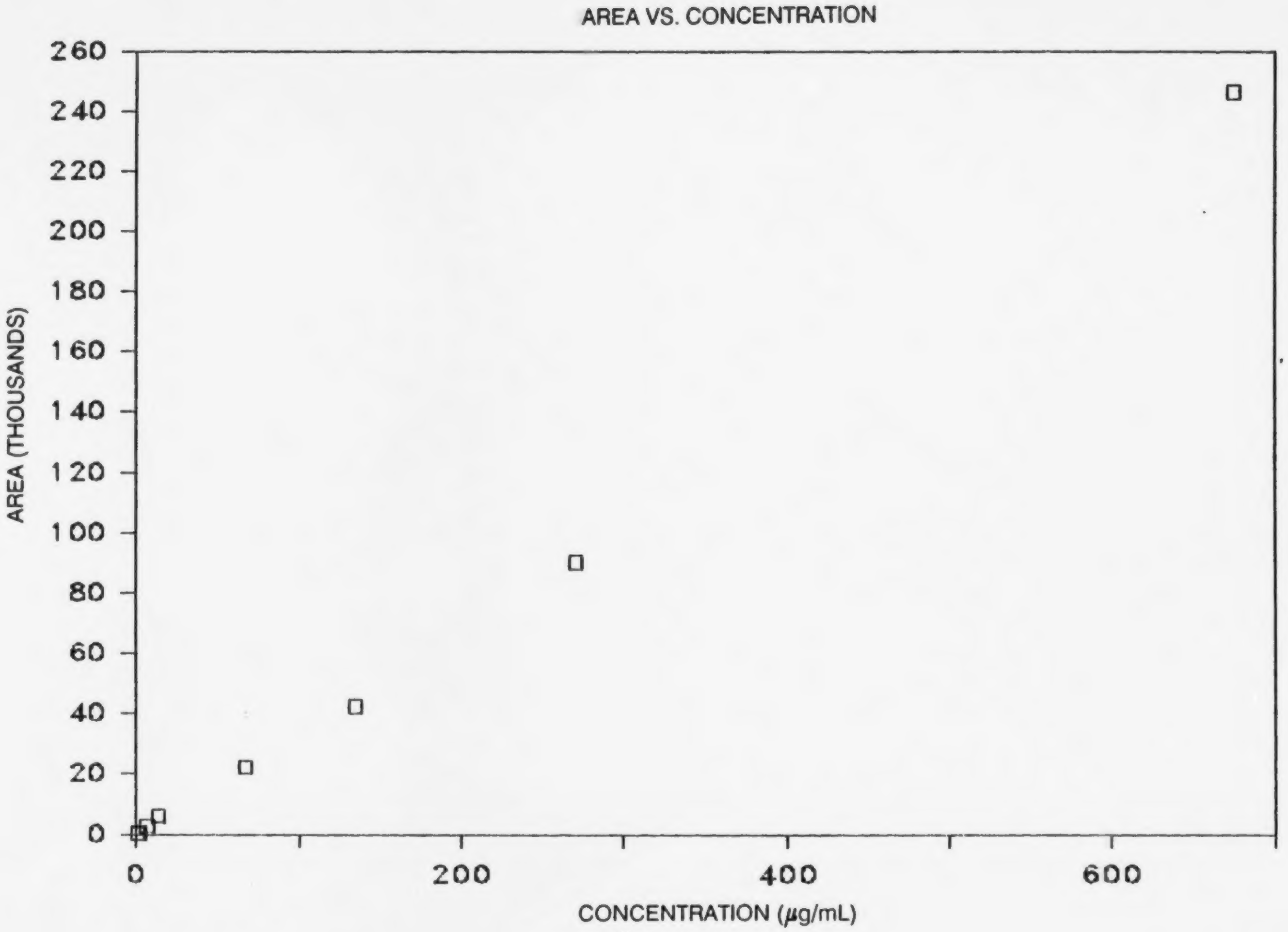


Figure 16B. Ethyl parathion: Response (arbitrary units) vs. concentration on the FPD detector.

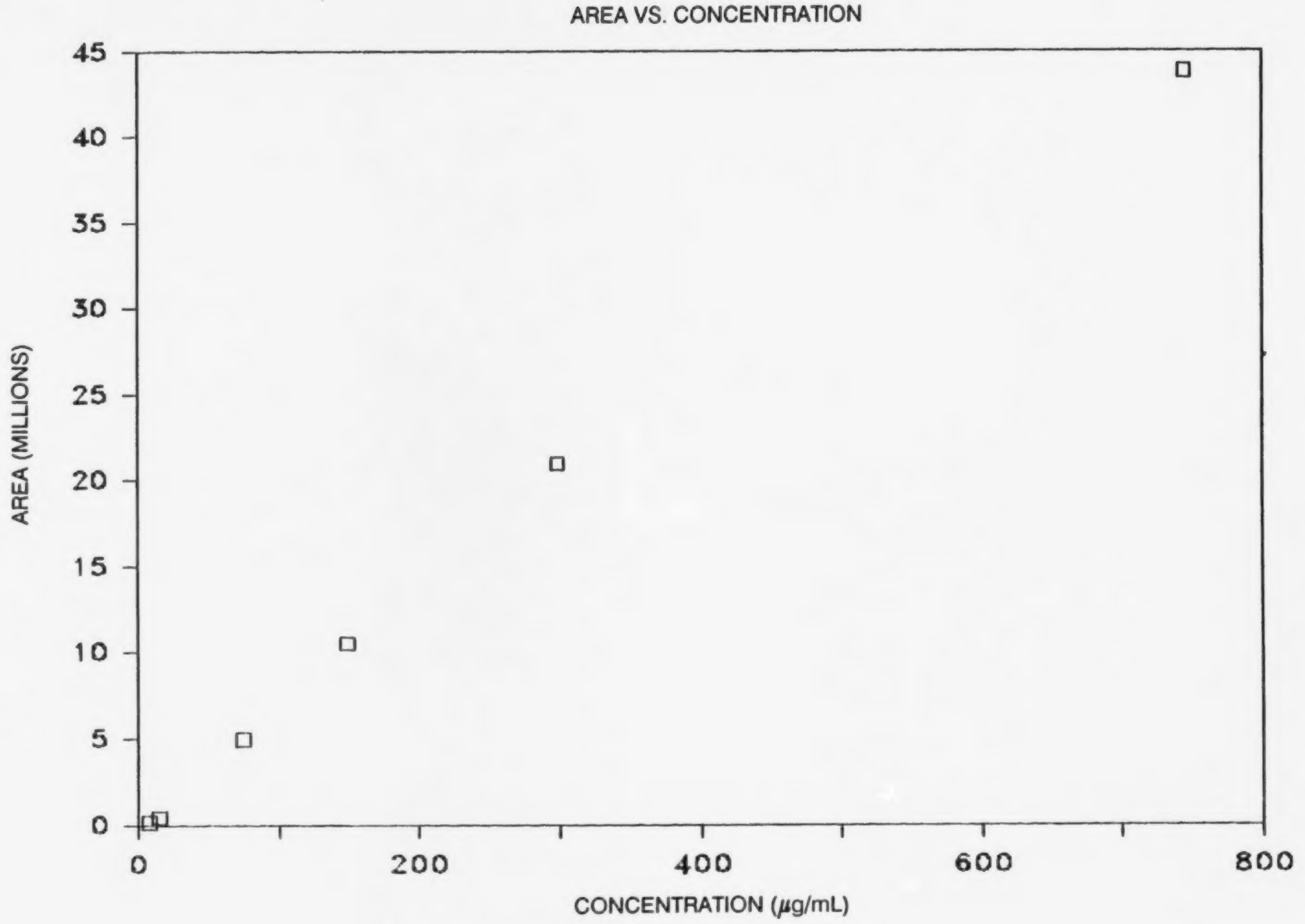


Figure 17A. Fenthion: Response (arbitrary units) vs. concentration on the NPD detector.

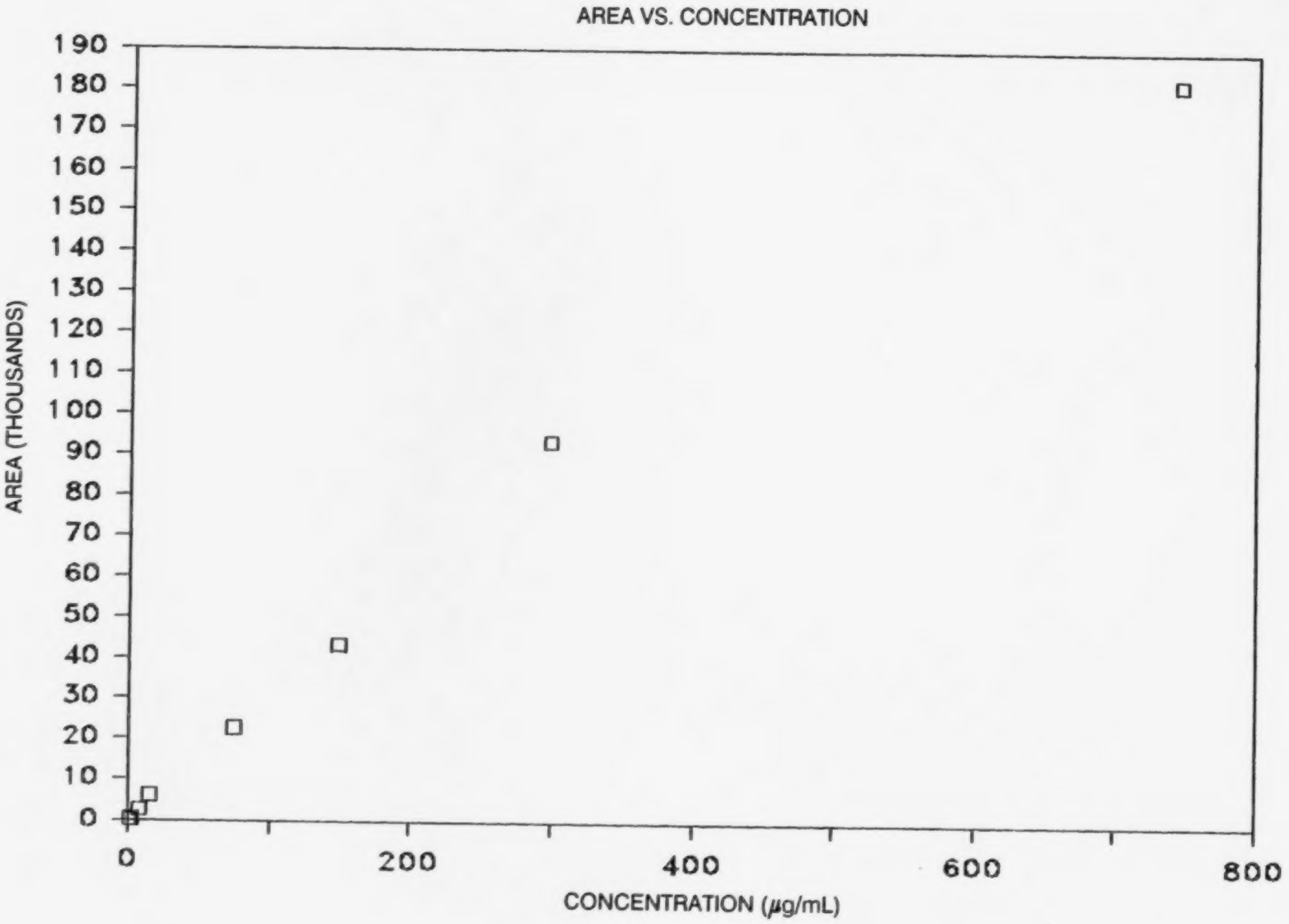


Figure 17B. Fenthion: Response (arbitrary units) vs. concentration on the FPD detector.

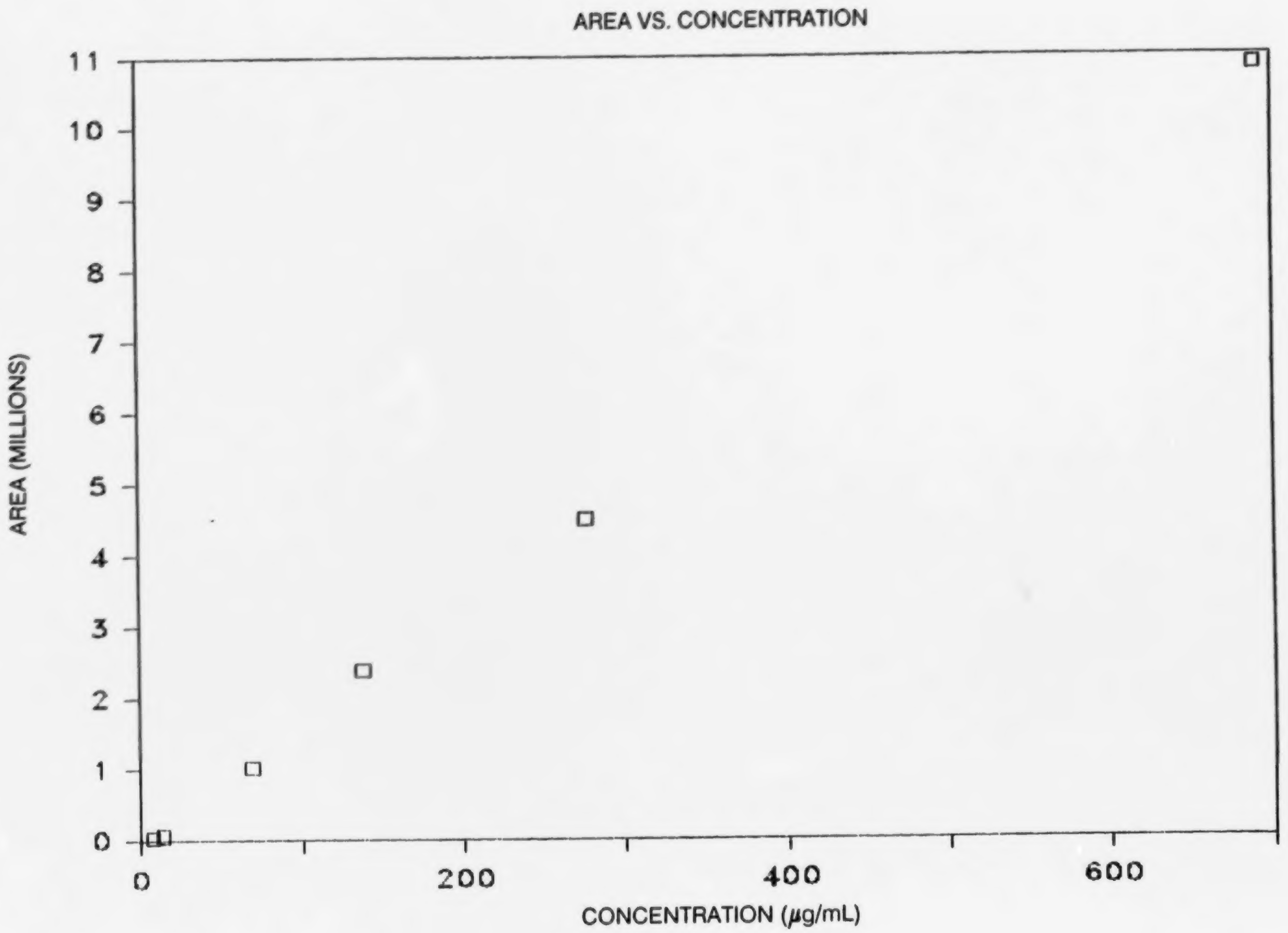


Figure 18A. Fensulfothion: Response (arbitrary units) vs. concentration on the NPD detector.

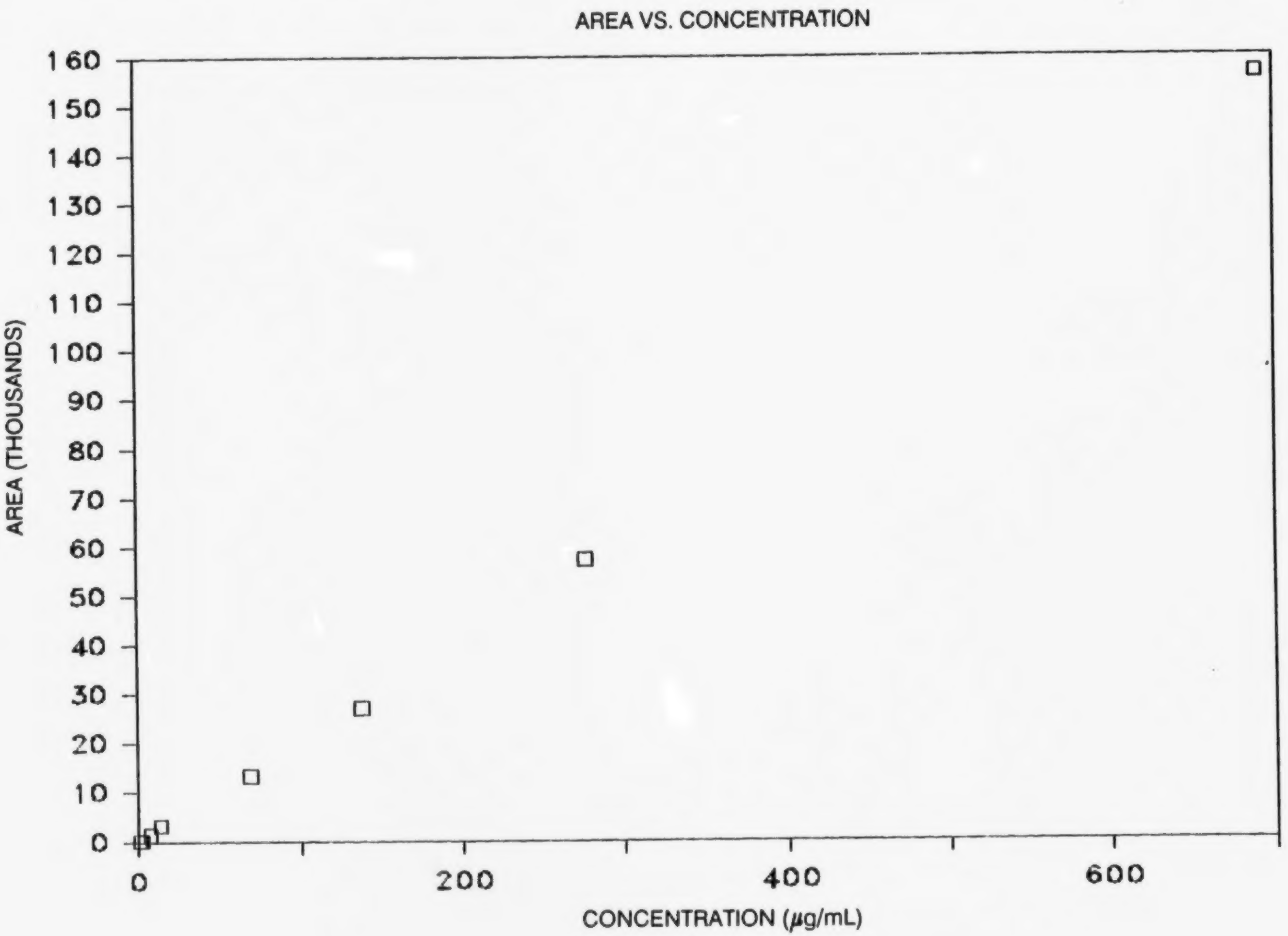


Figure 18B. Fensulfothion: Response (arbitrary units) vs. concentration on the FPD detector.

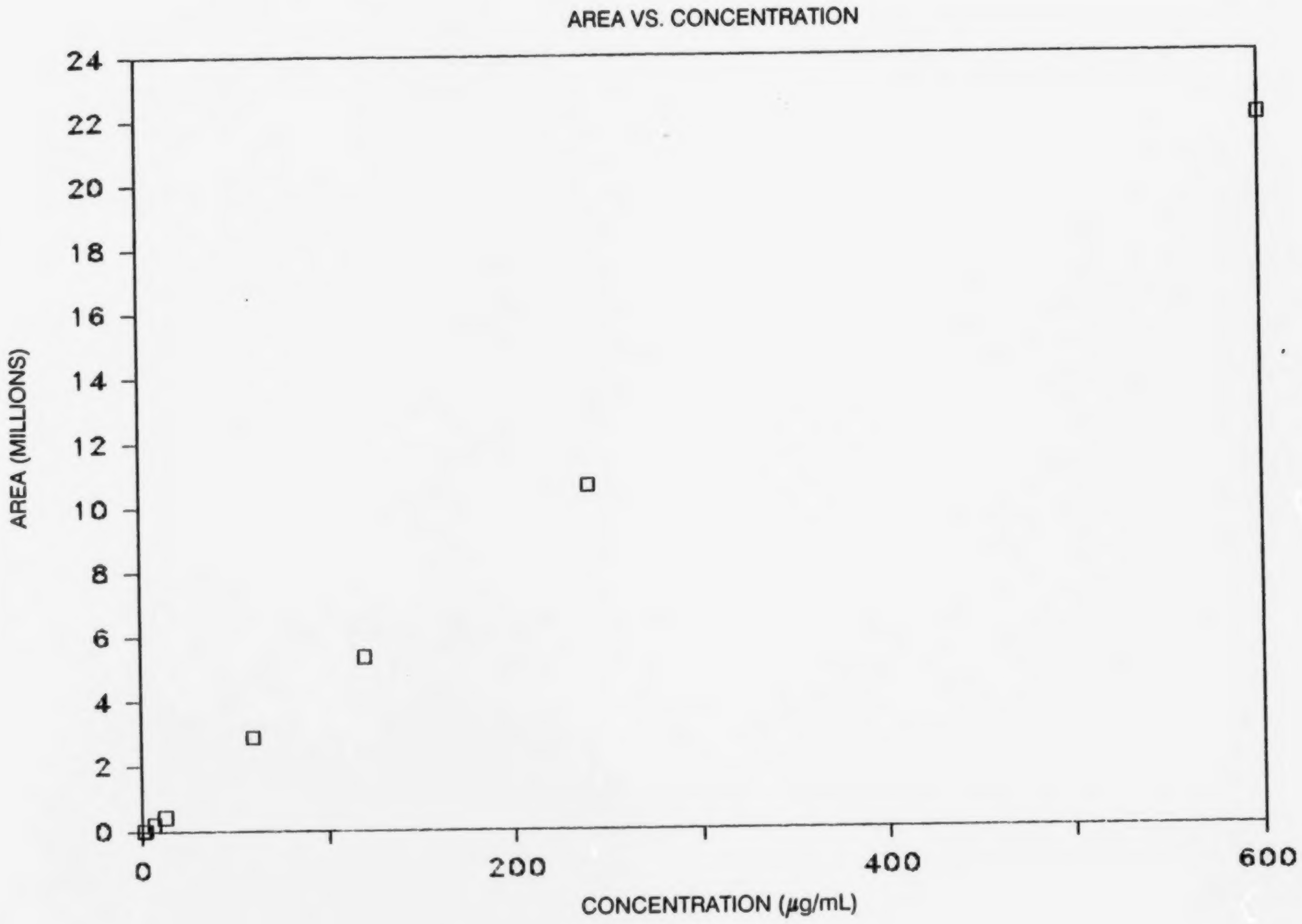


Figure 19A. Malathion: Response (arbitrary units) vs. concentration on the NPD detector.

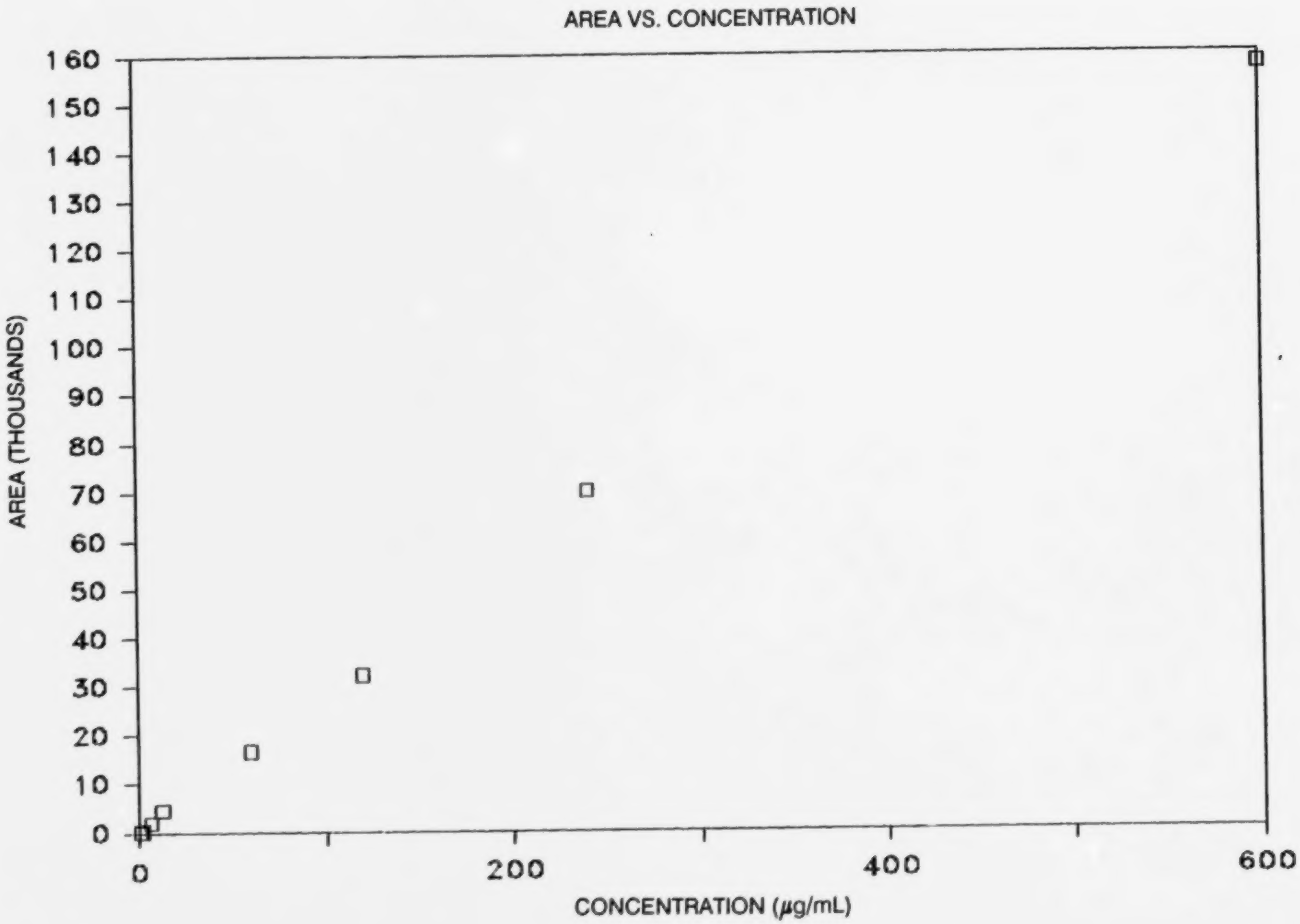


Figure 19B. **Malathion: Response (arbitrary units) vs. concentration on the FPD detector.**

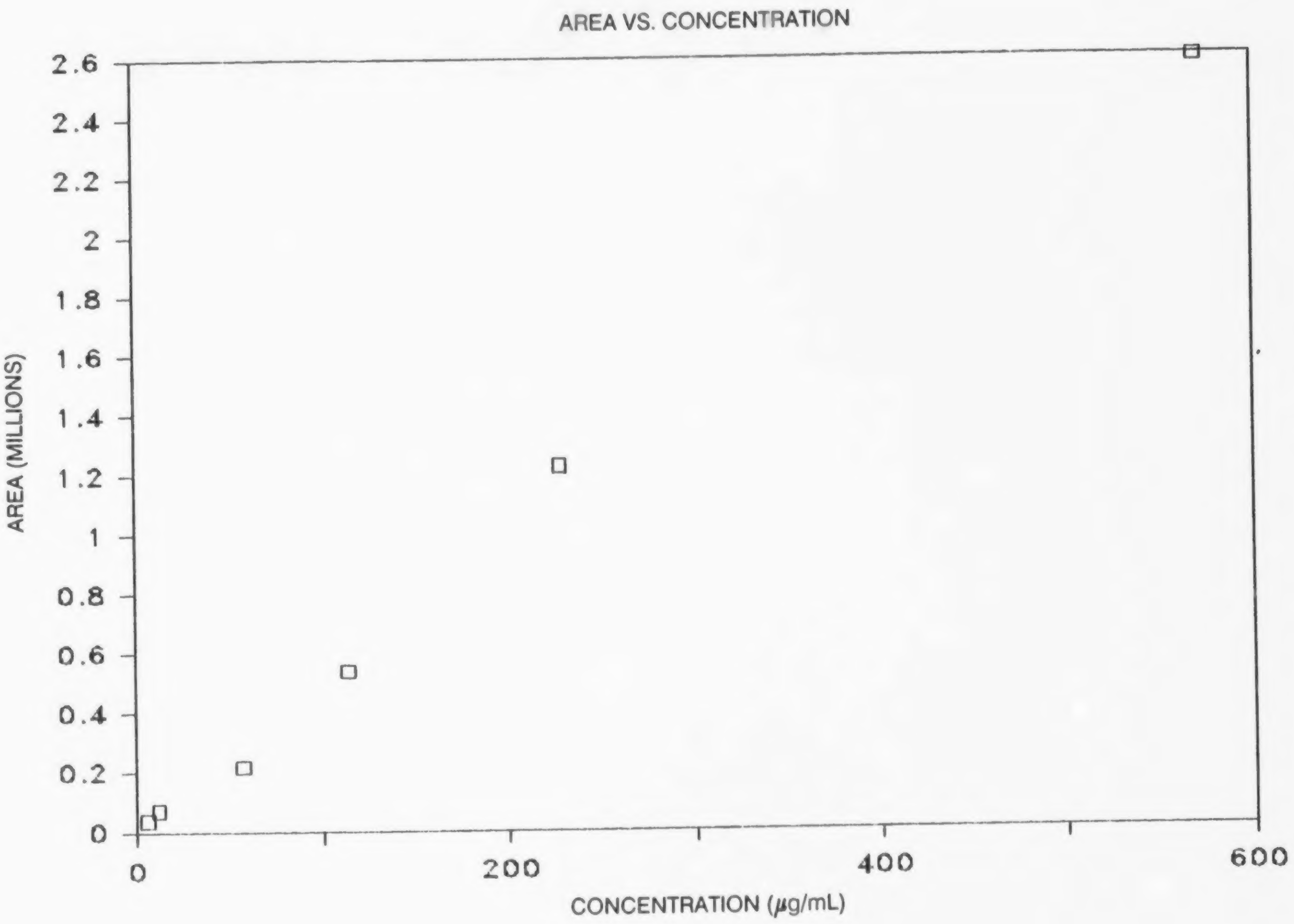


Figure 20A. Merphos: Response (arbitrary units) vs. concentration on the NPD detector.

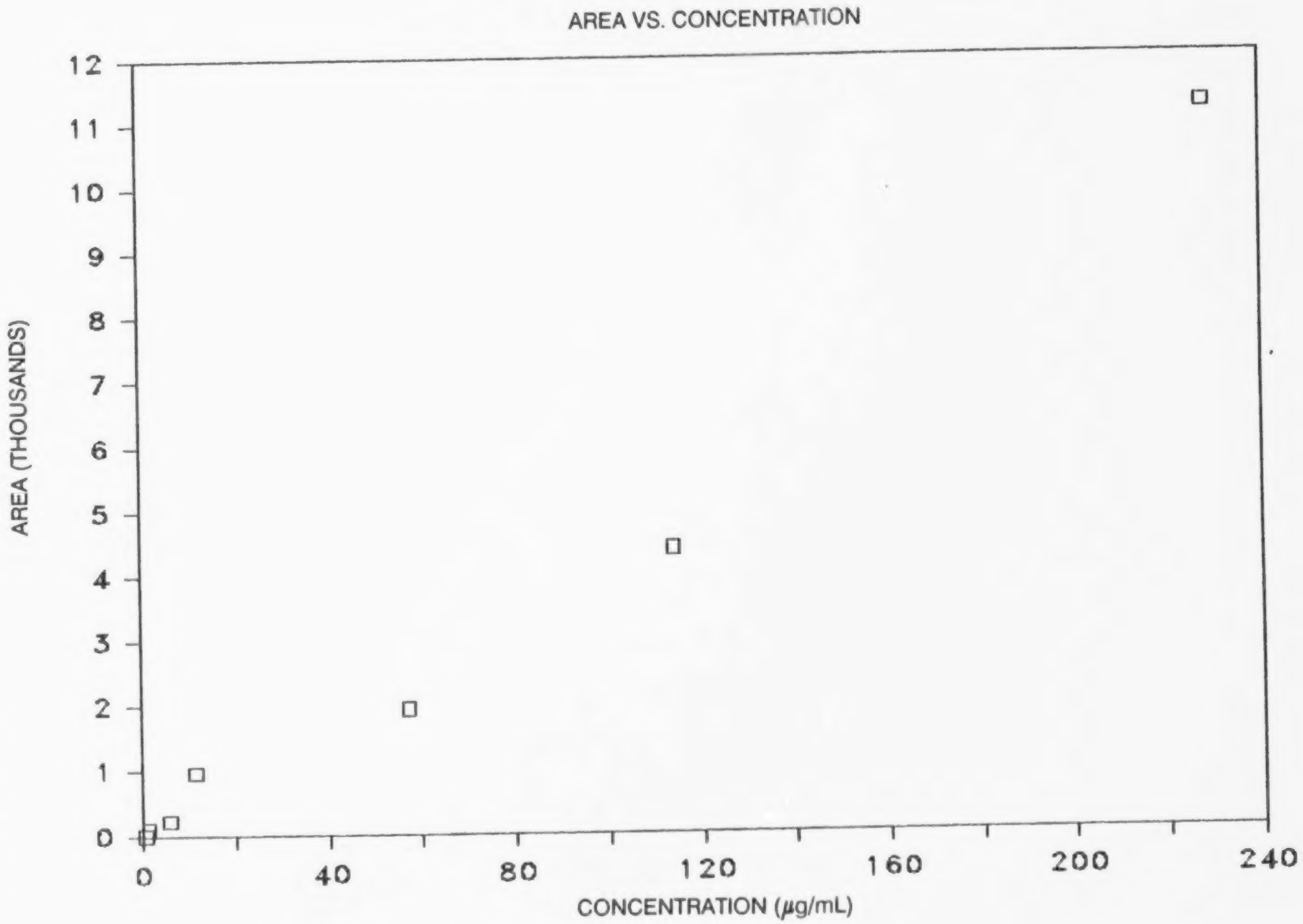


Figure 20B. **Merphos: Response (arbitrary units) vs. concentration on the FPD detector.**

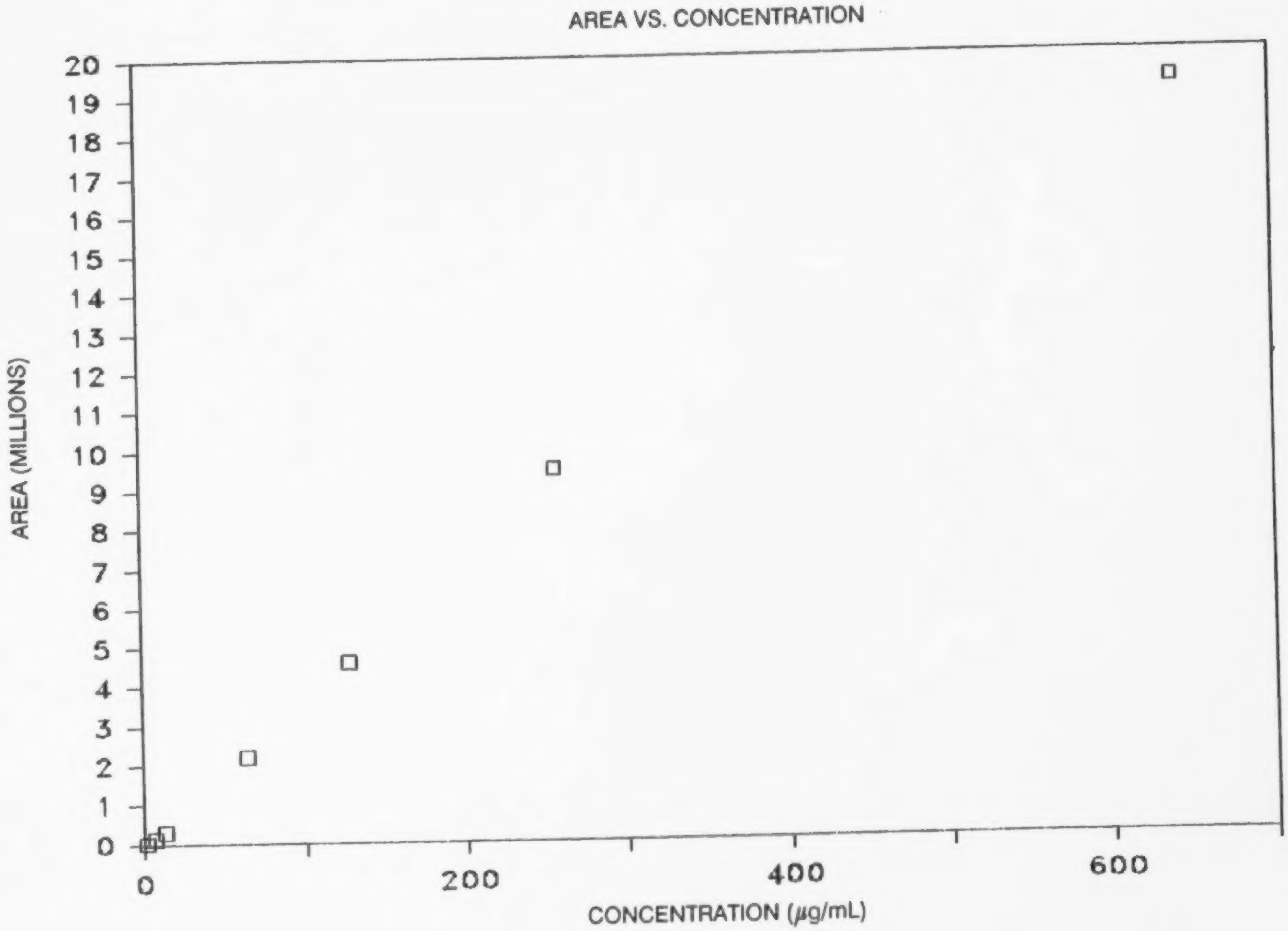


Figure 21A. Methyl parathion: Response (arbitrary units) vs. concentration on the NPD detector.

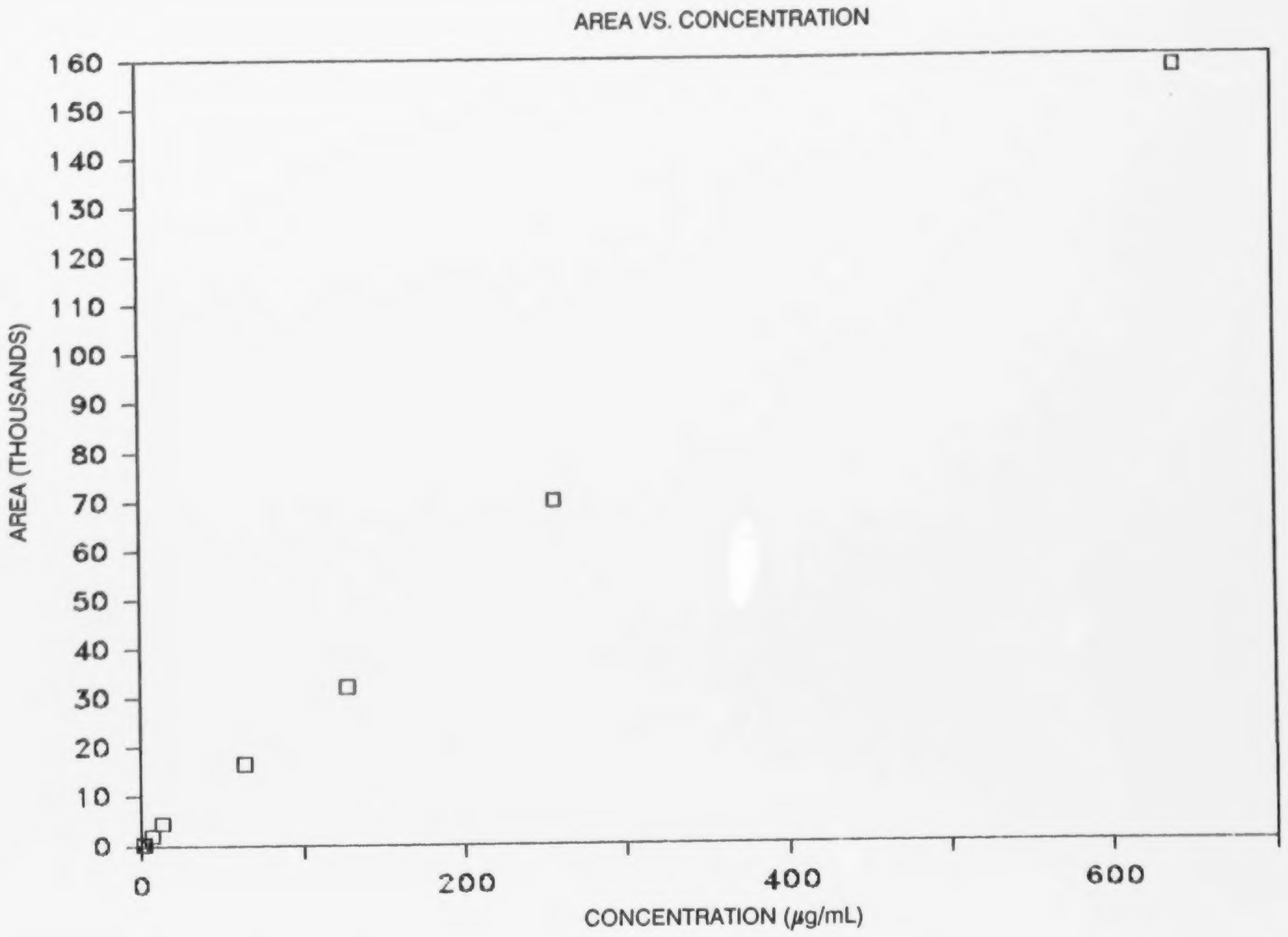


Figure 21B. Methyl parathion: Response (arbitrary units) vs. concentration on the FPD detector.

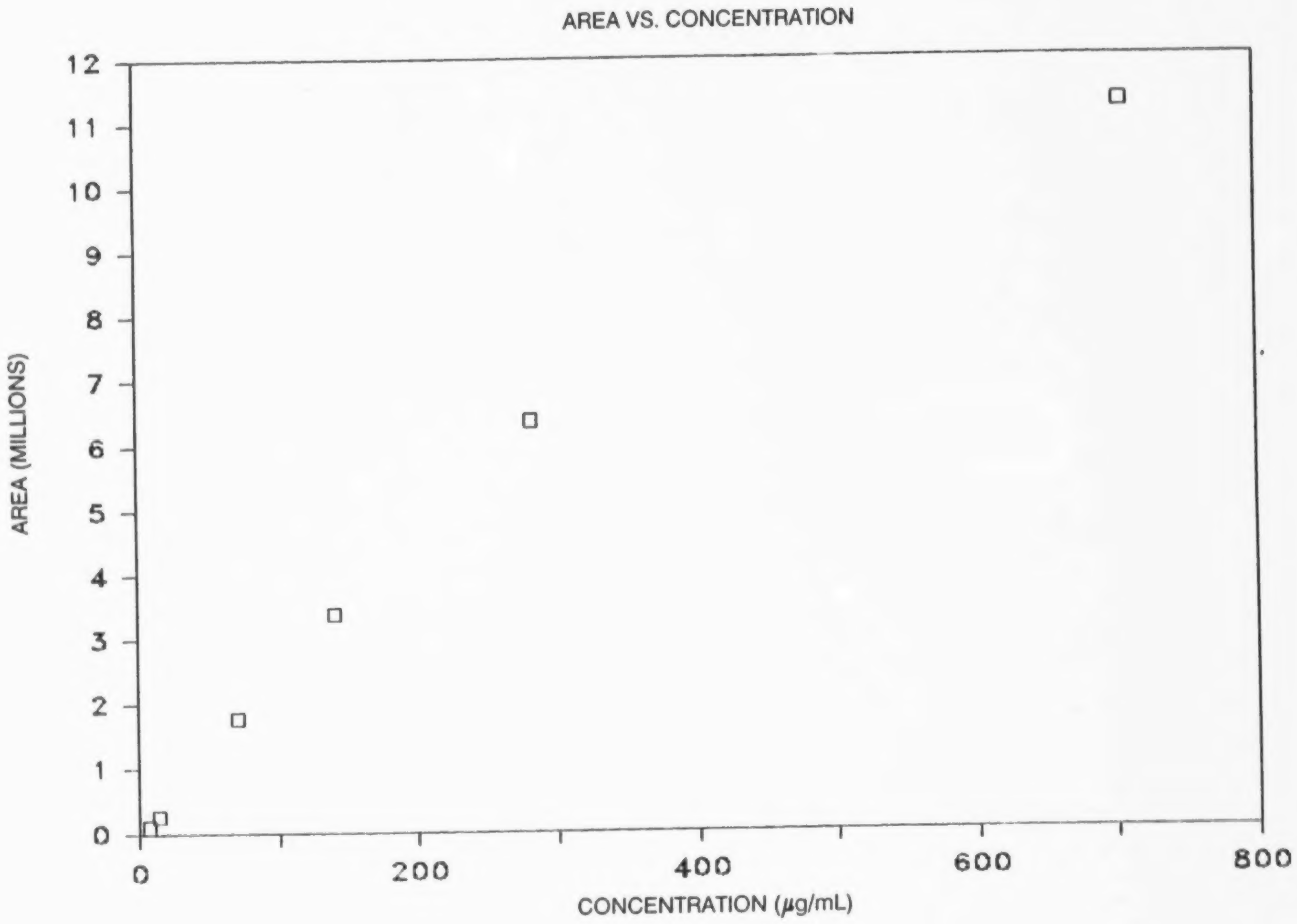


Figure 22A. Mevinphos: Response (arbitrary units) vs. concentration on the NPD detector.

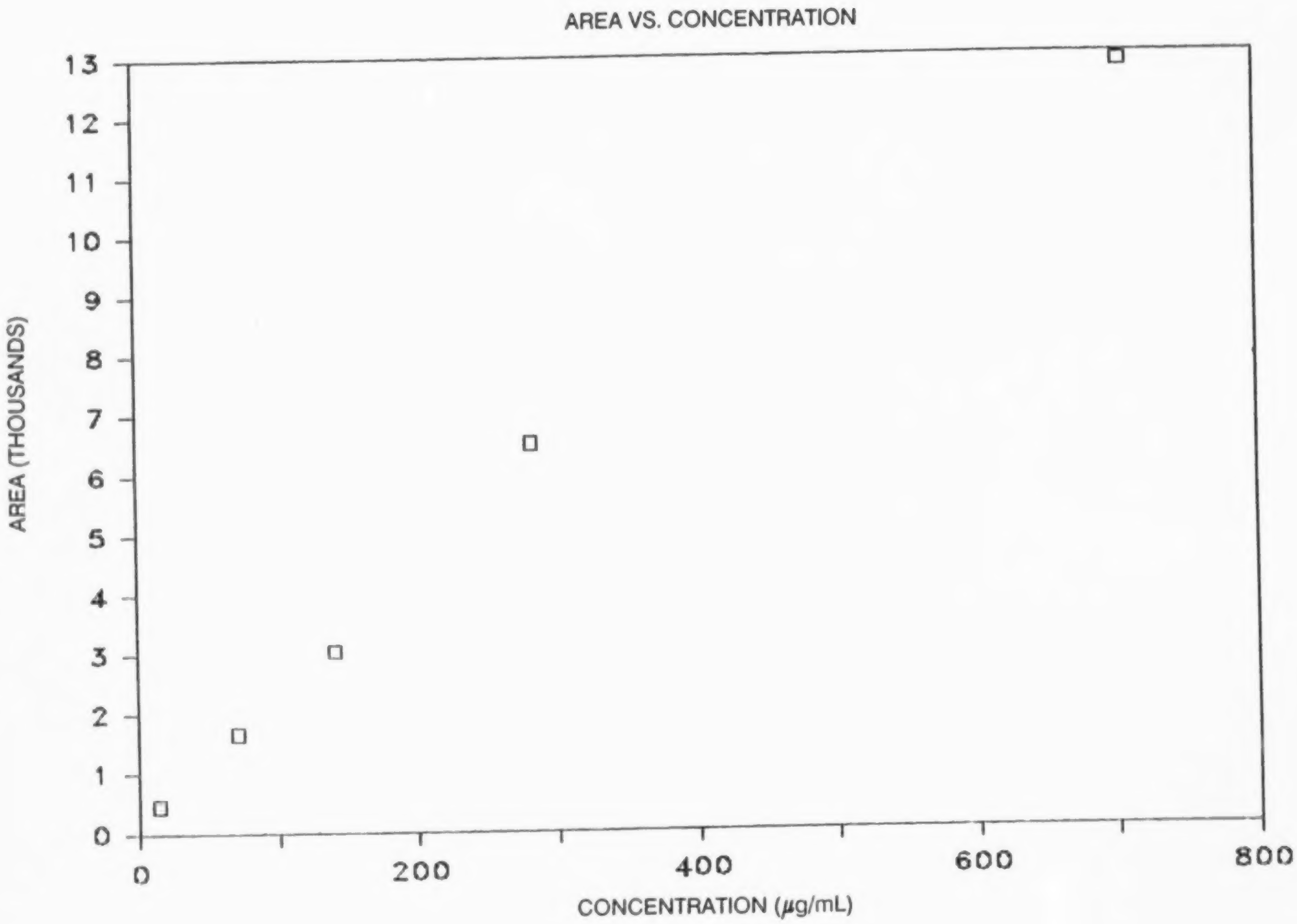


Figure 22B. Mevinphos: Response (arbitrary units) vs. concentration on the FPD detector.

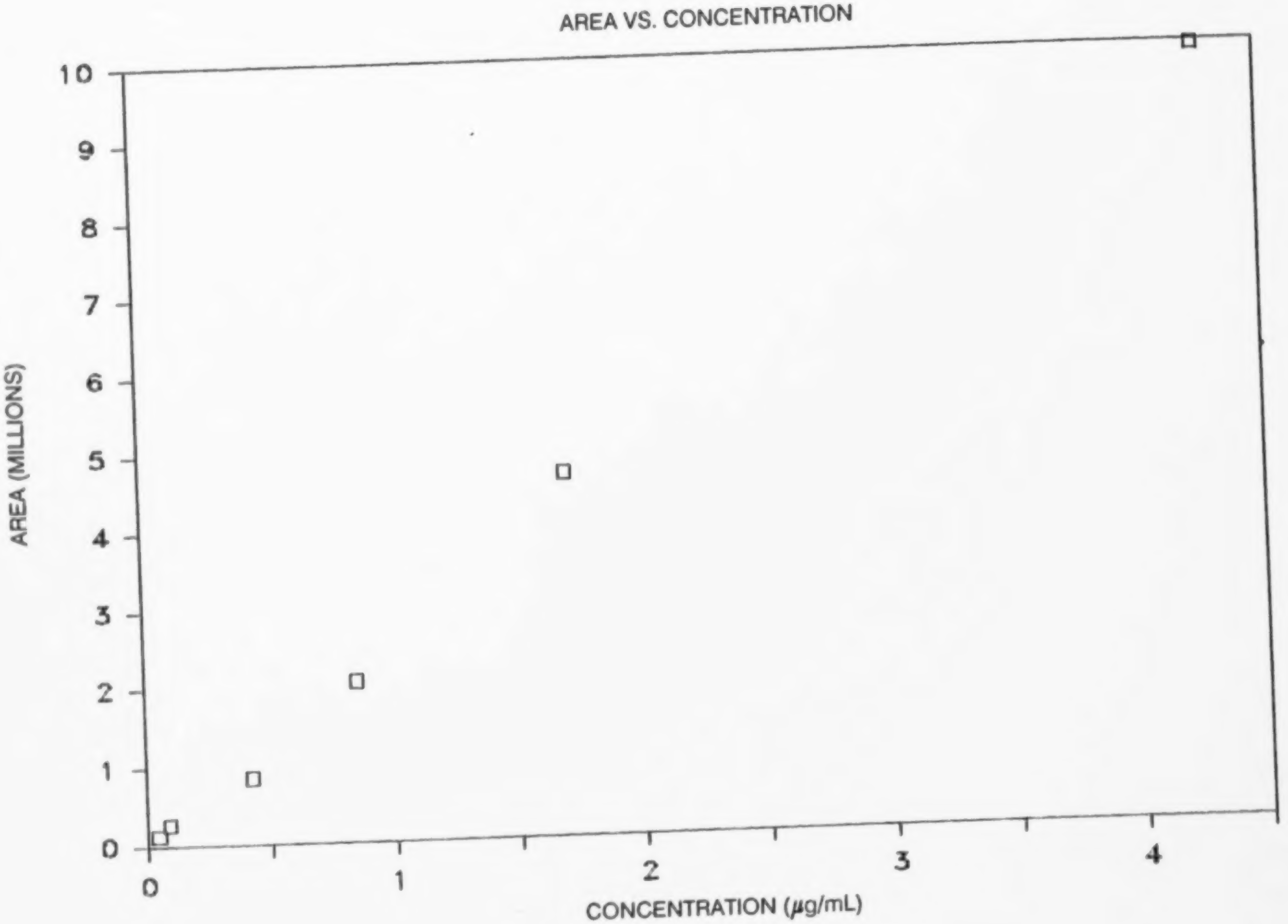


Figure 23A. Monocrotophos: Response (arbitrary units) vs. concentration on the NPD detector.

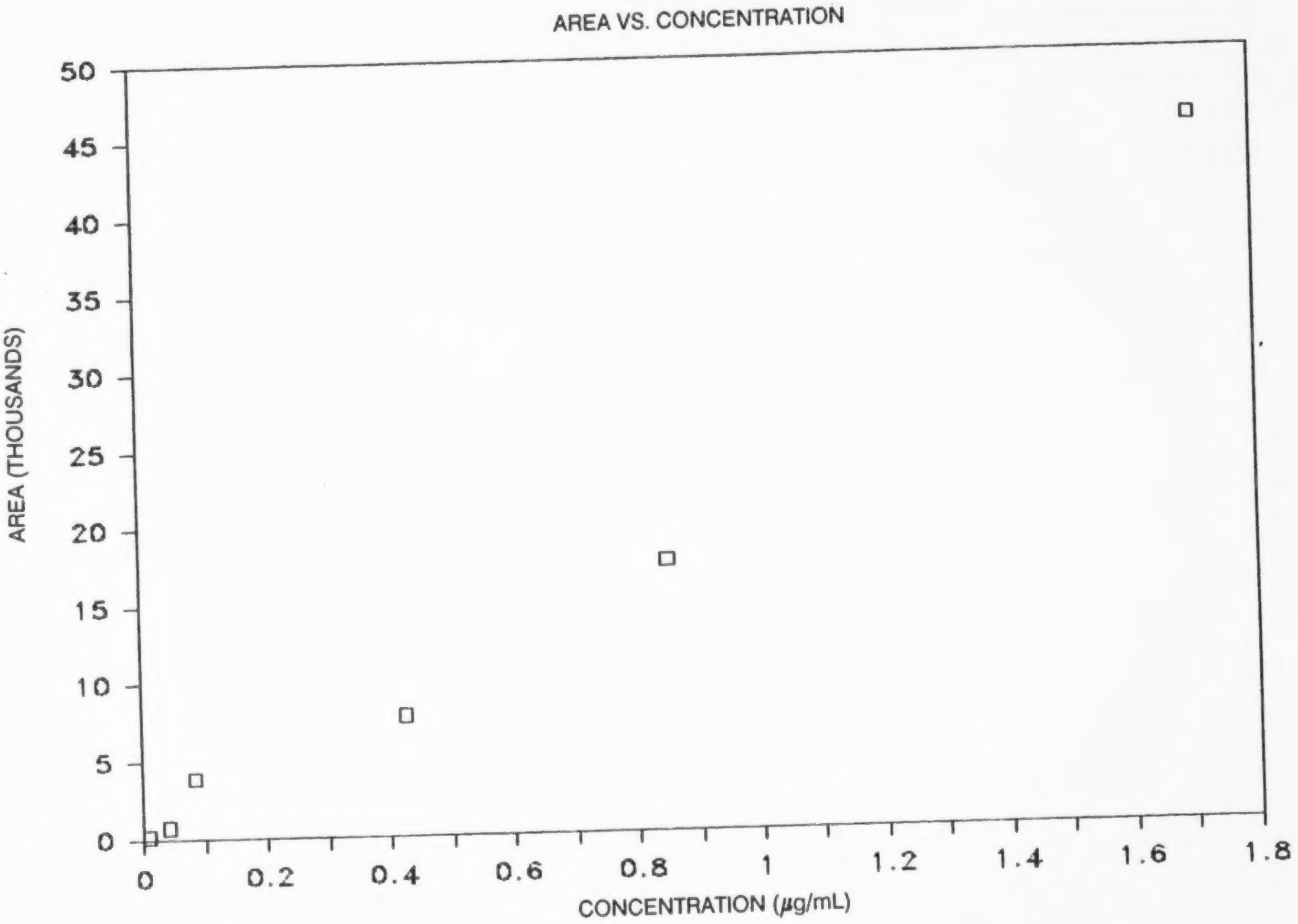


Figure 23B. **Monocrotophos: Response (arbitrary units) vs. concentration on the FPD detector.**

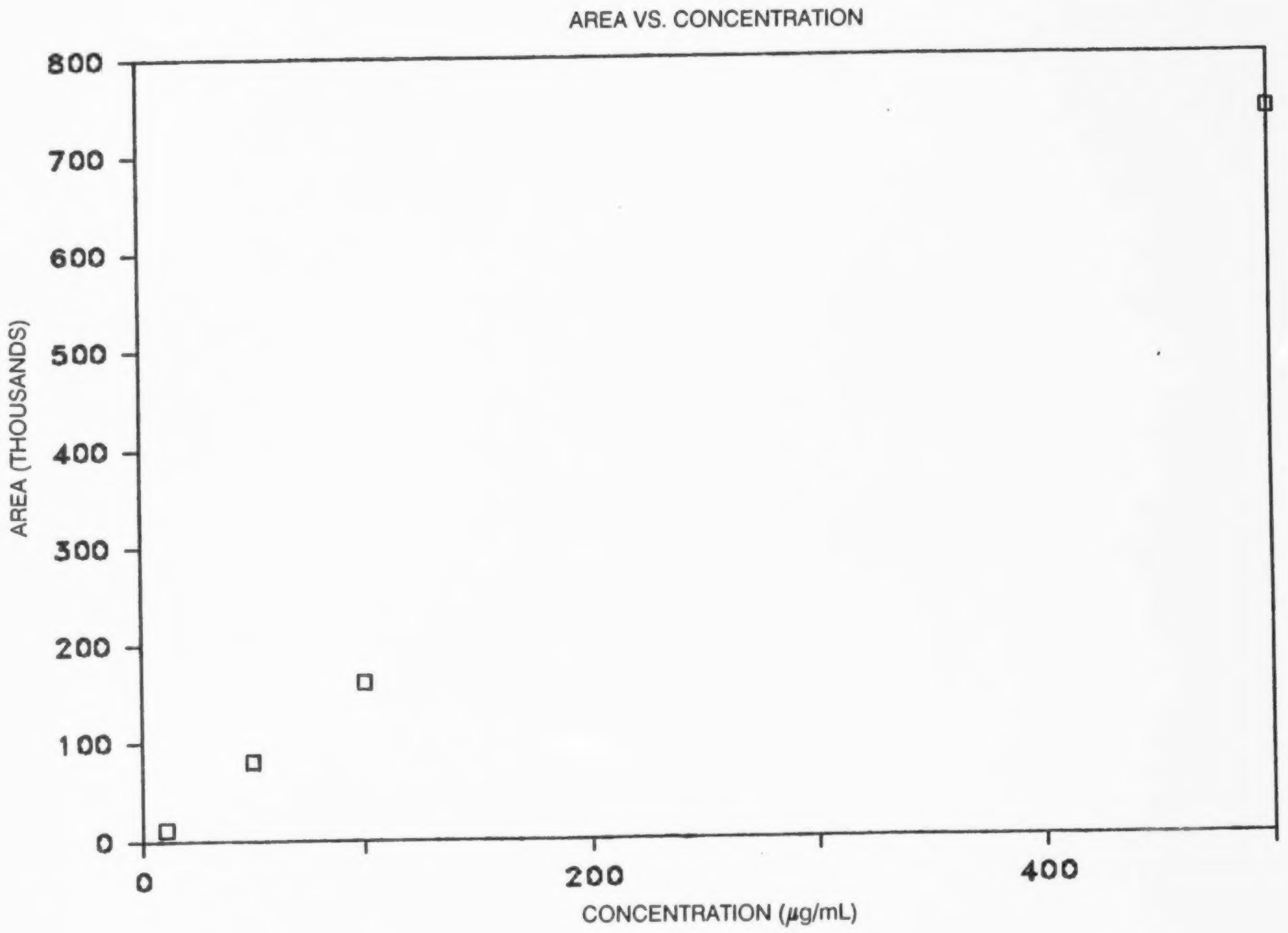


Figure 24. Naled: Response (arbitrary units) vs. concentration on the FPD detector.

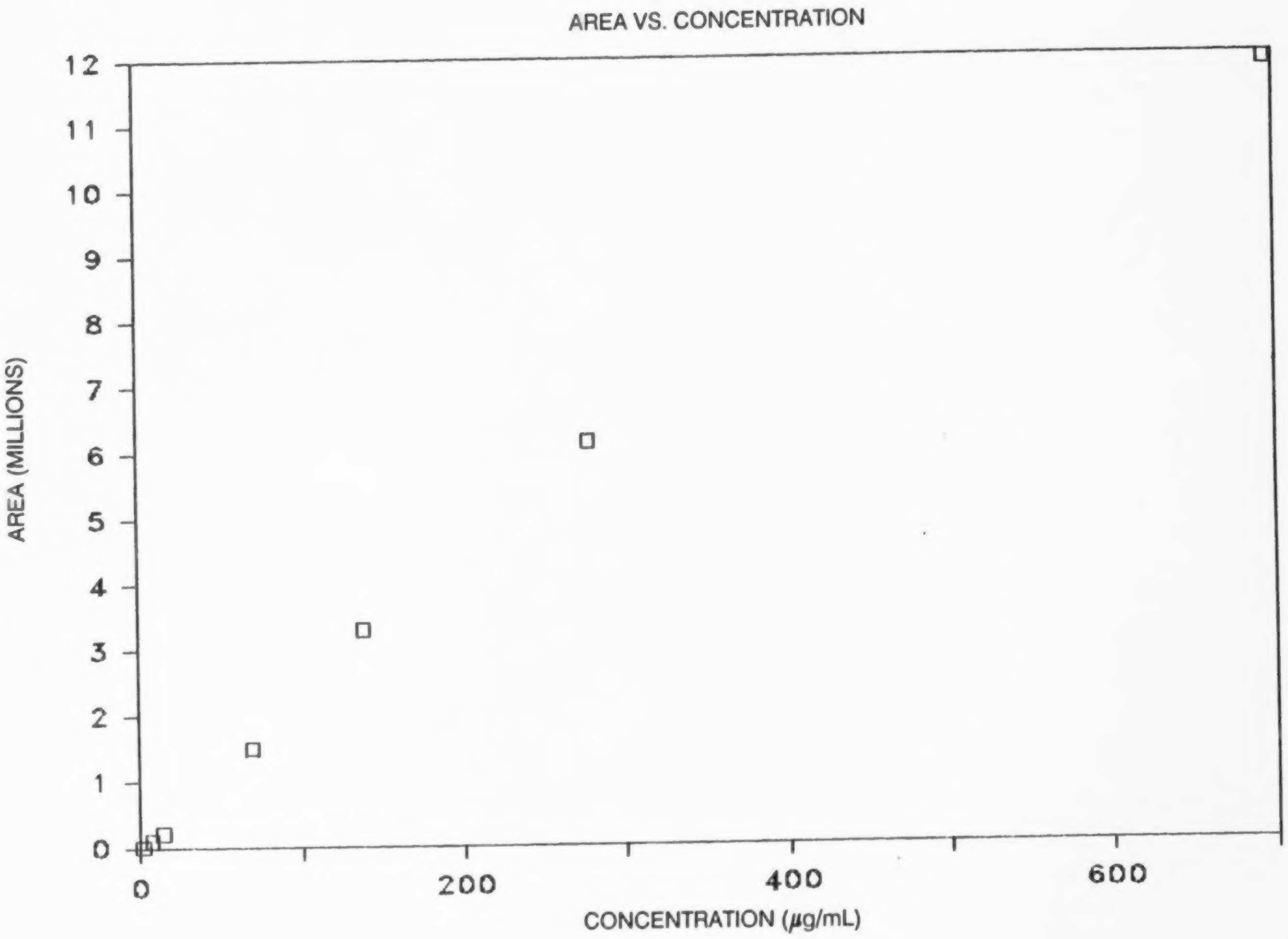


Figure 25A. **Phorate: Response (arbitrary units) vs. concentration on the NPD detector.**

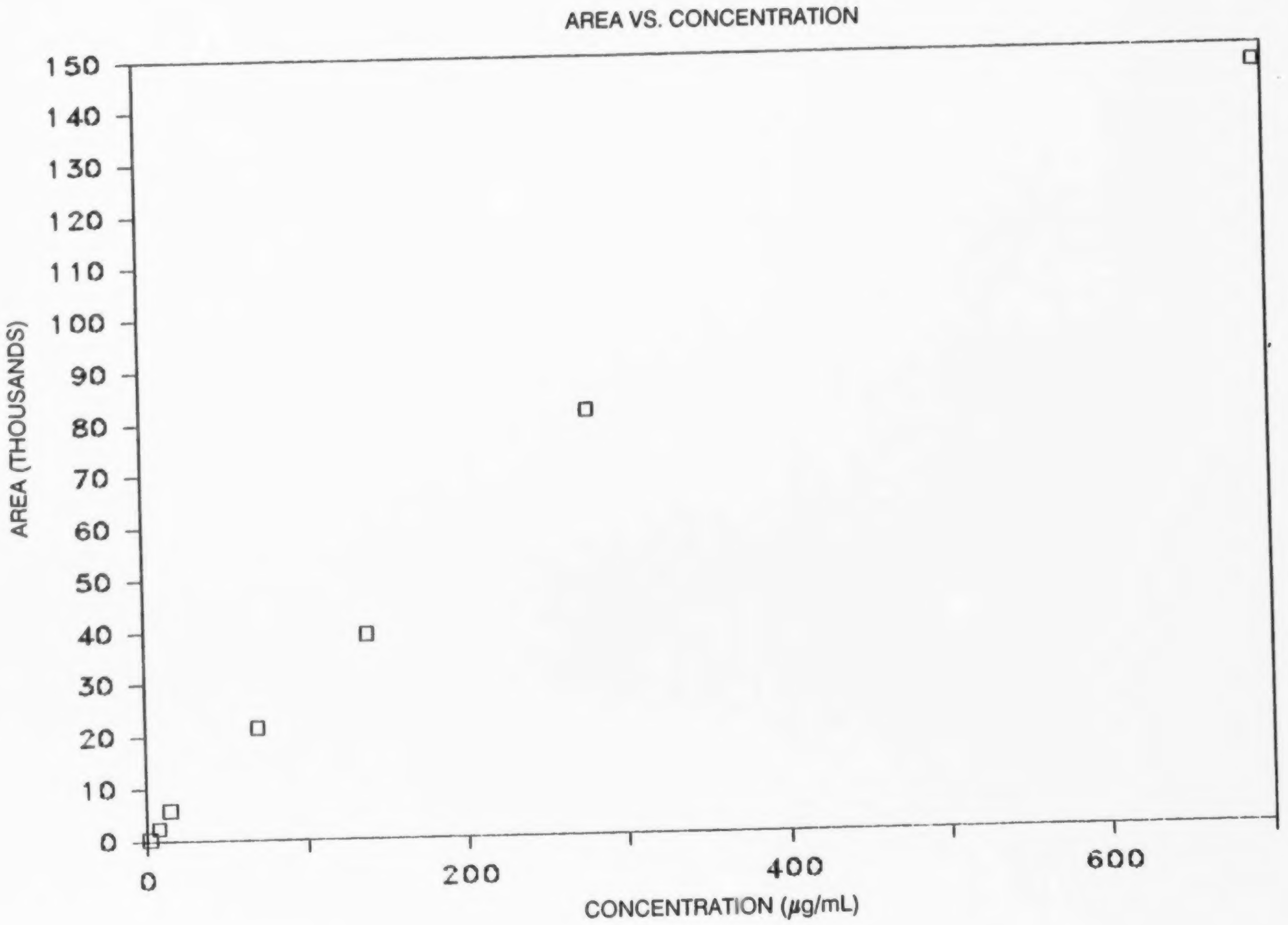


Figure 25B. Phorate: Response (arbitrary units) vs. concentration on the FPD detector.

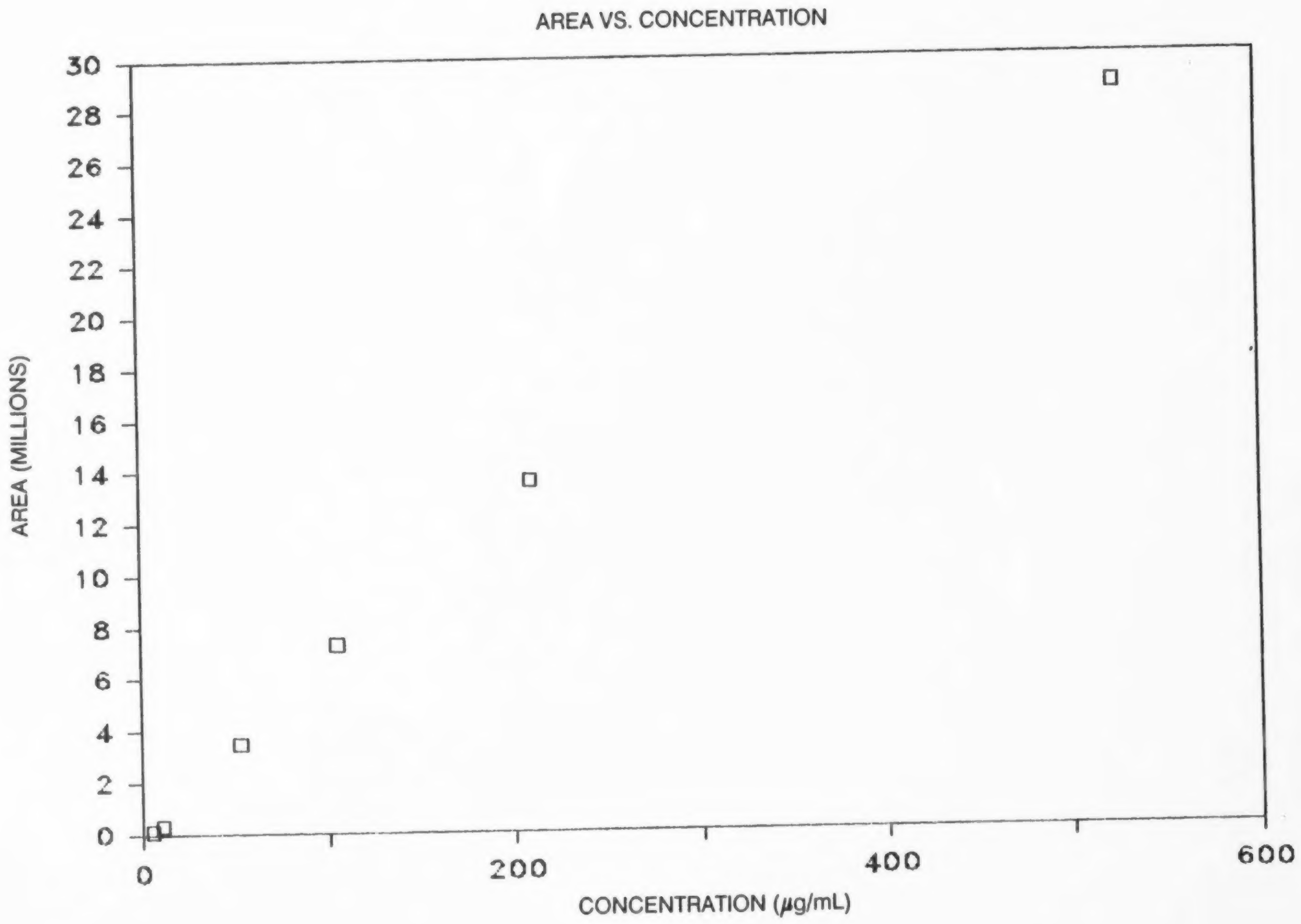


Figure 26A. Ronnel: Response (arbitrary units) vs. concentration on the NPD detector.

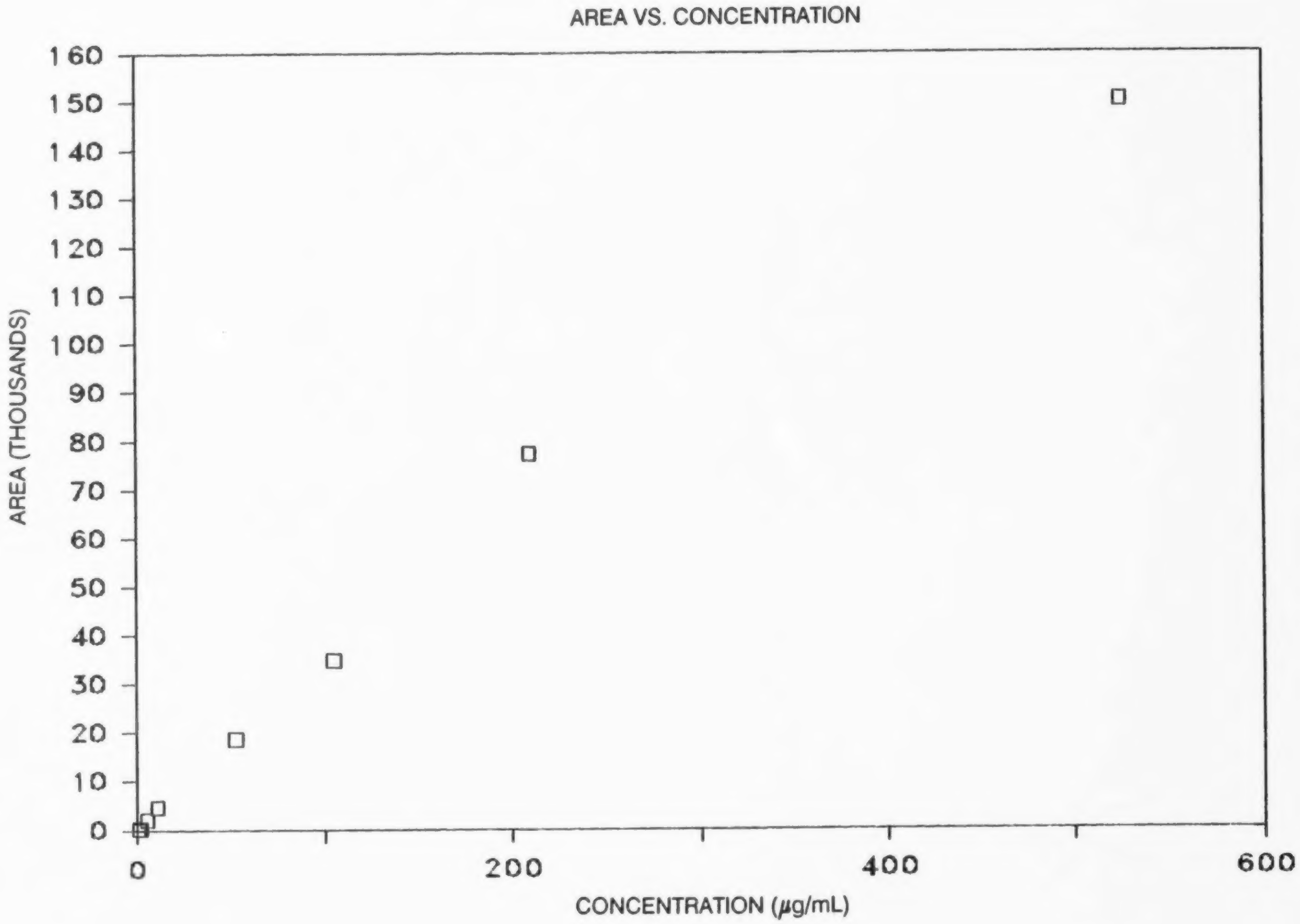


Figure 26B. Ronnel: Response (arbitrary units) vs. concentration on the FPD detector.

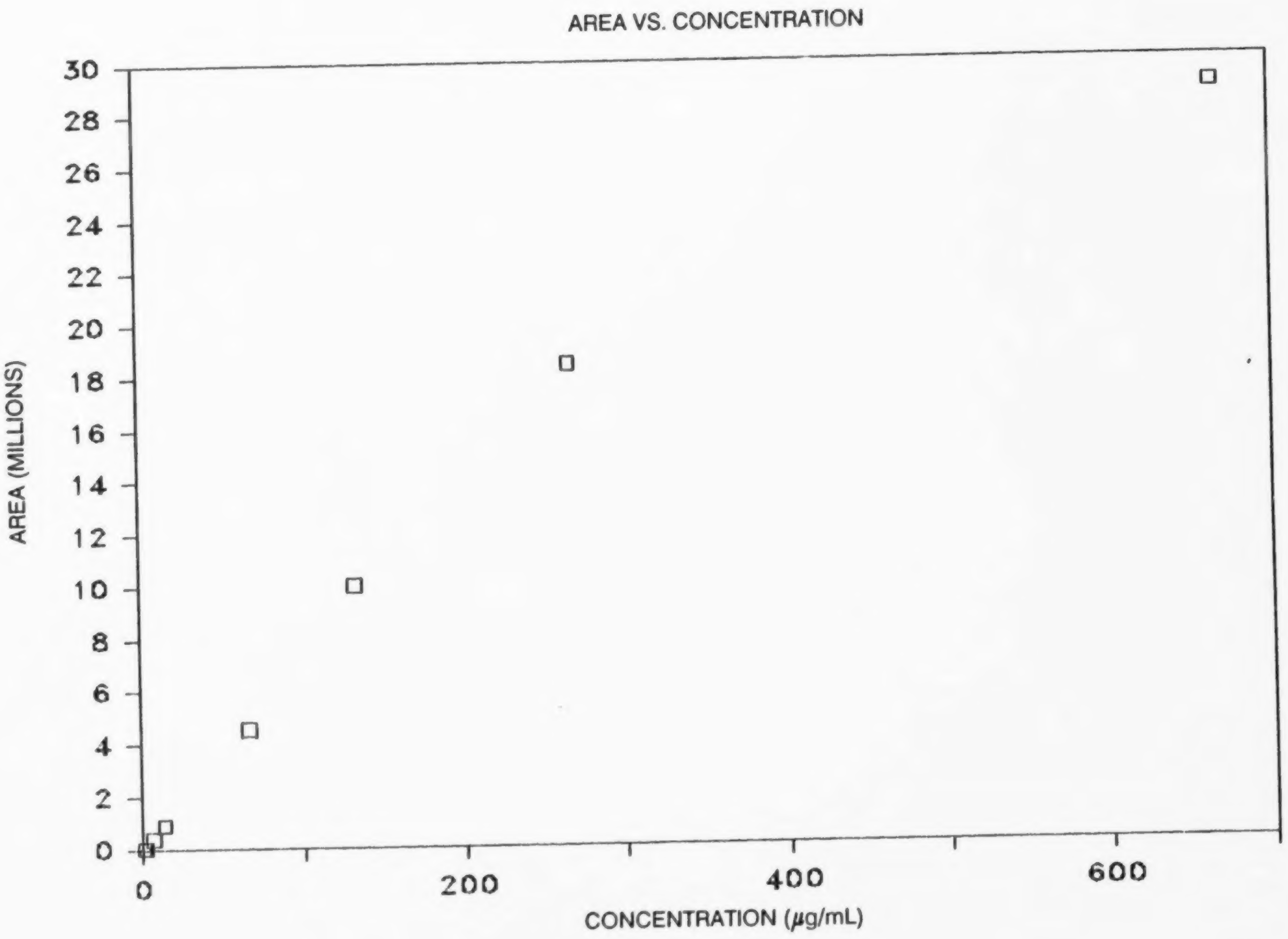


Figure 27A. Sulfotep: Response (arbitrary units) vs. concentration on the NPD detector.

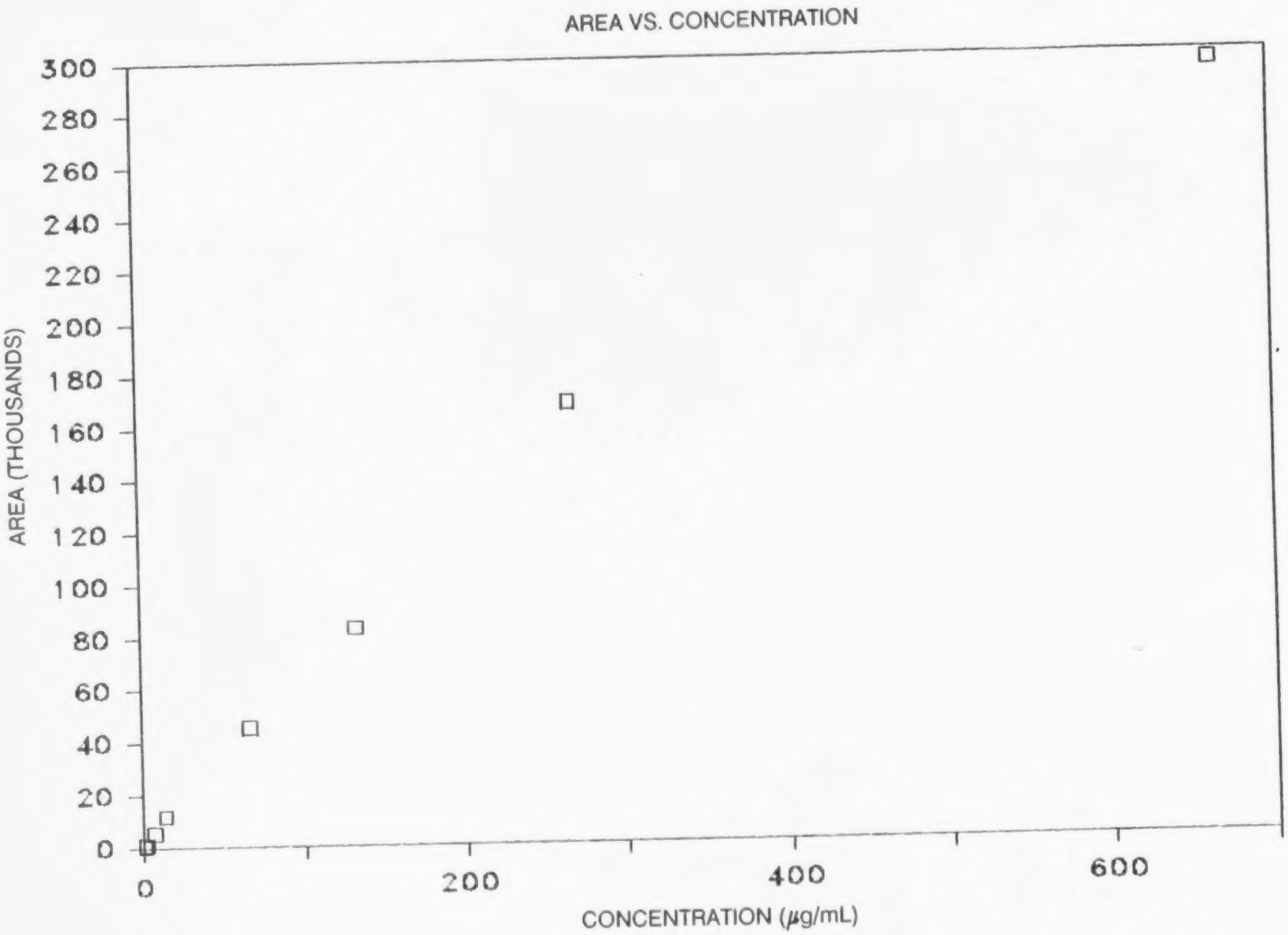


Figure 27B. Sulfotep: Response (arbitrary units) vs. concentration on the FPD detector.

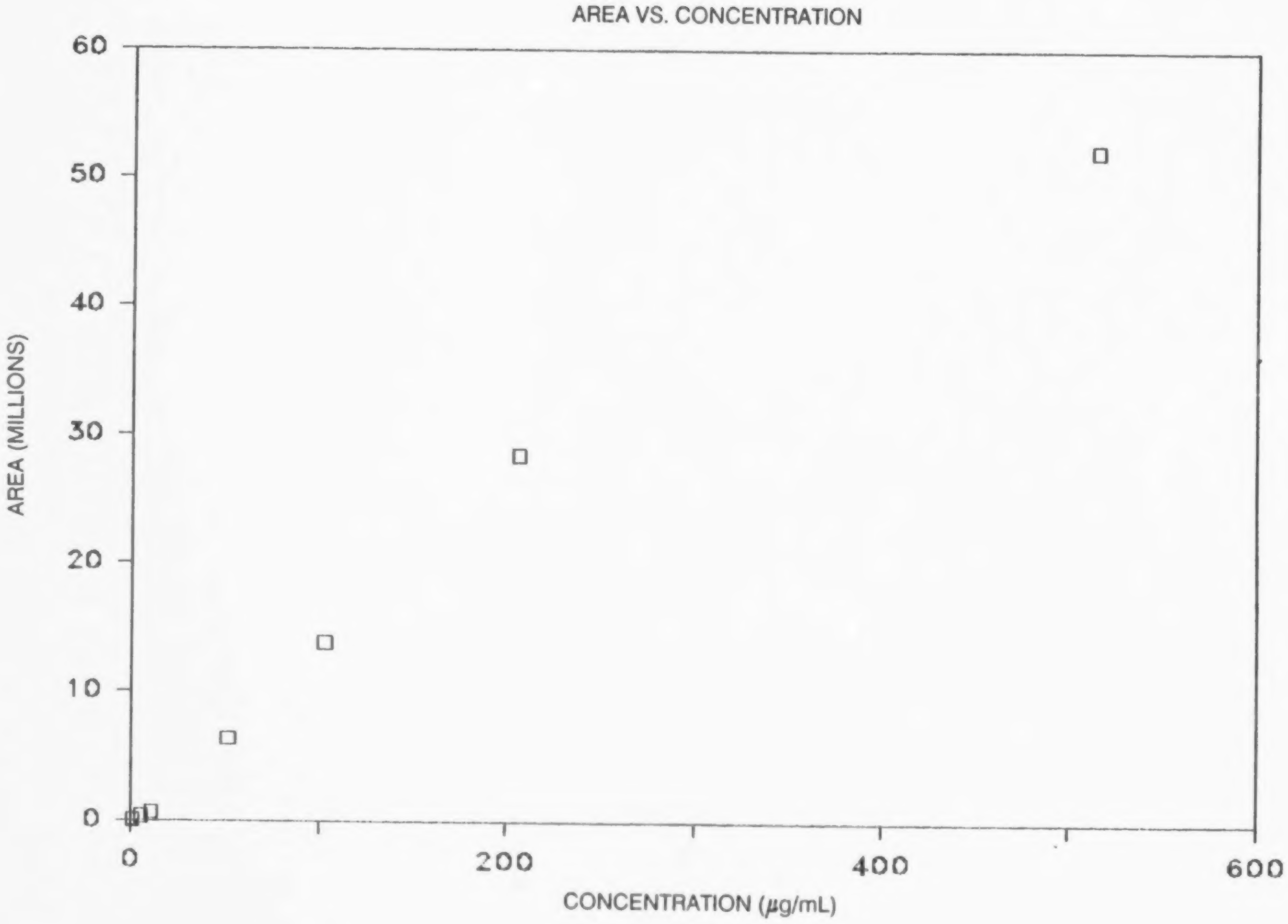


Figure 28A. Tetrachlorvinphos: Response (arbitrary units) vs. concentration on the FPD detector.

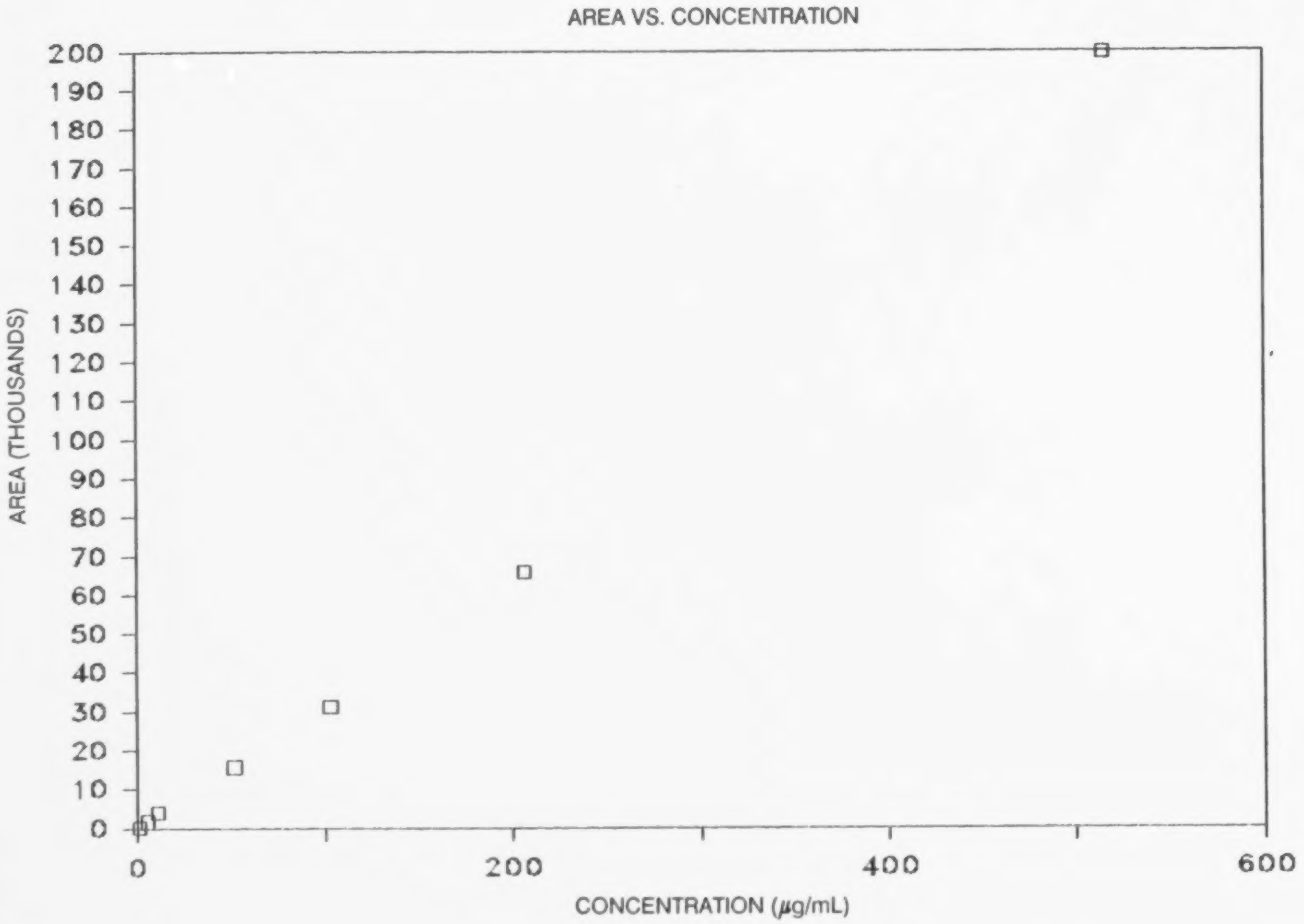


Figure 28B. Tetrachlorvinphos: Response (arbitrary units) vs. concentration on the FPD detector.

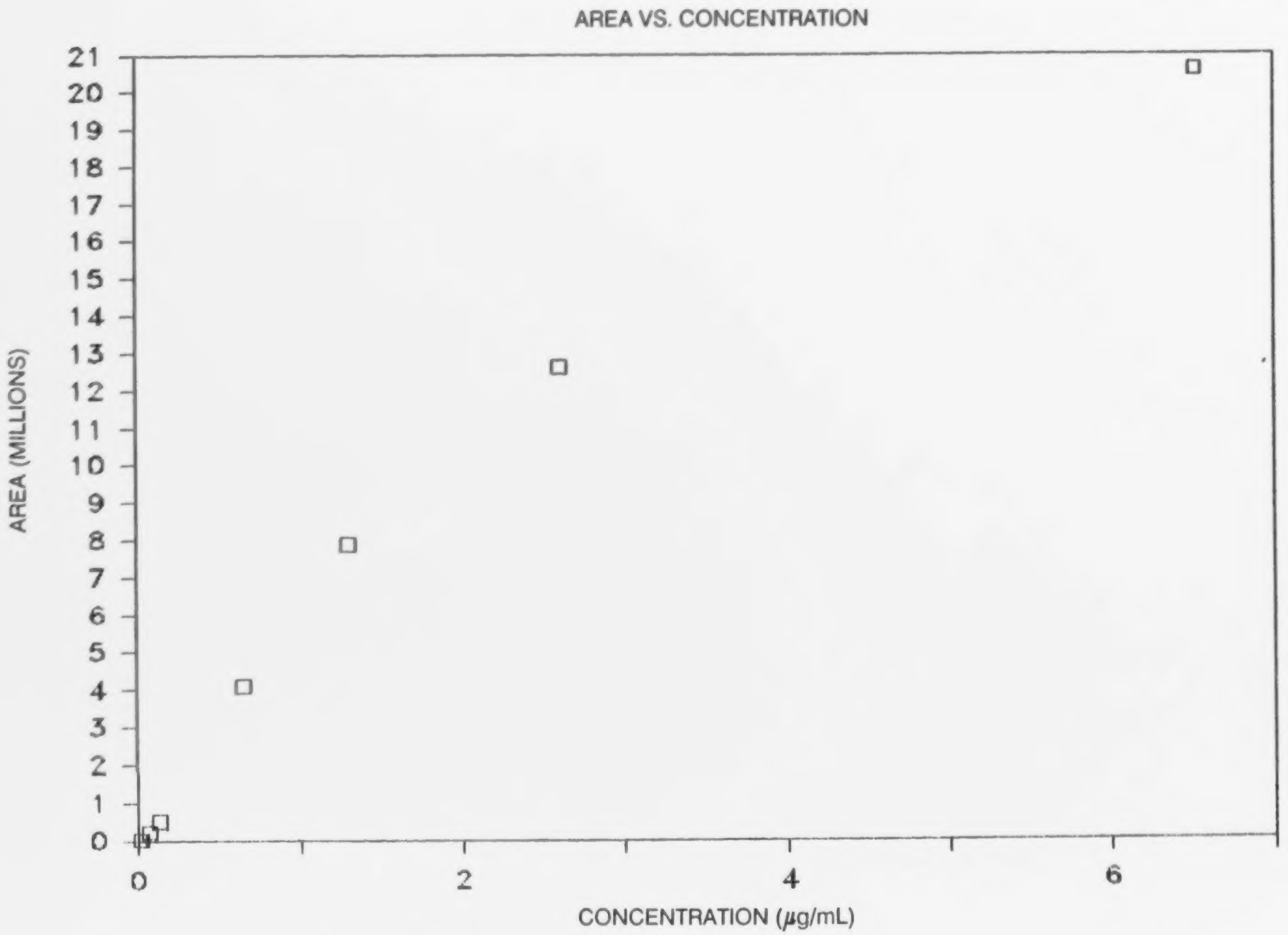


Figure 29A. TEPP: Response (arbitrary units) vs. concentration on the NPD detector.

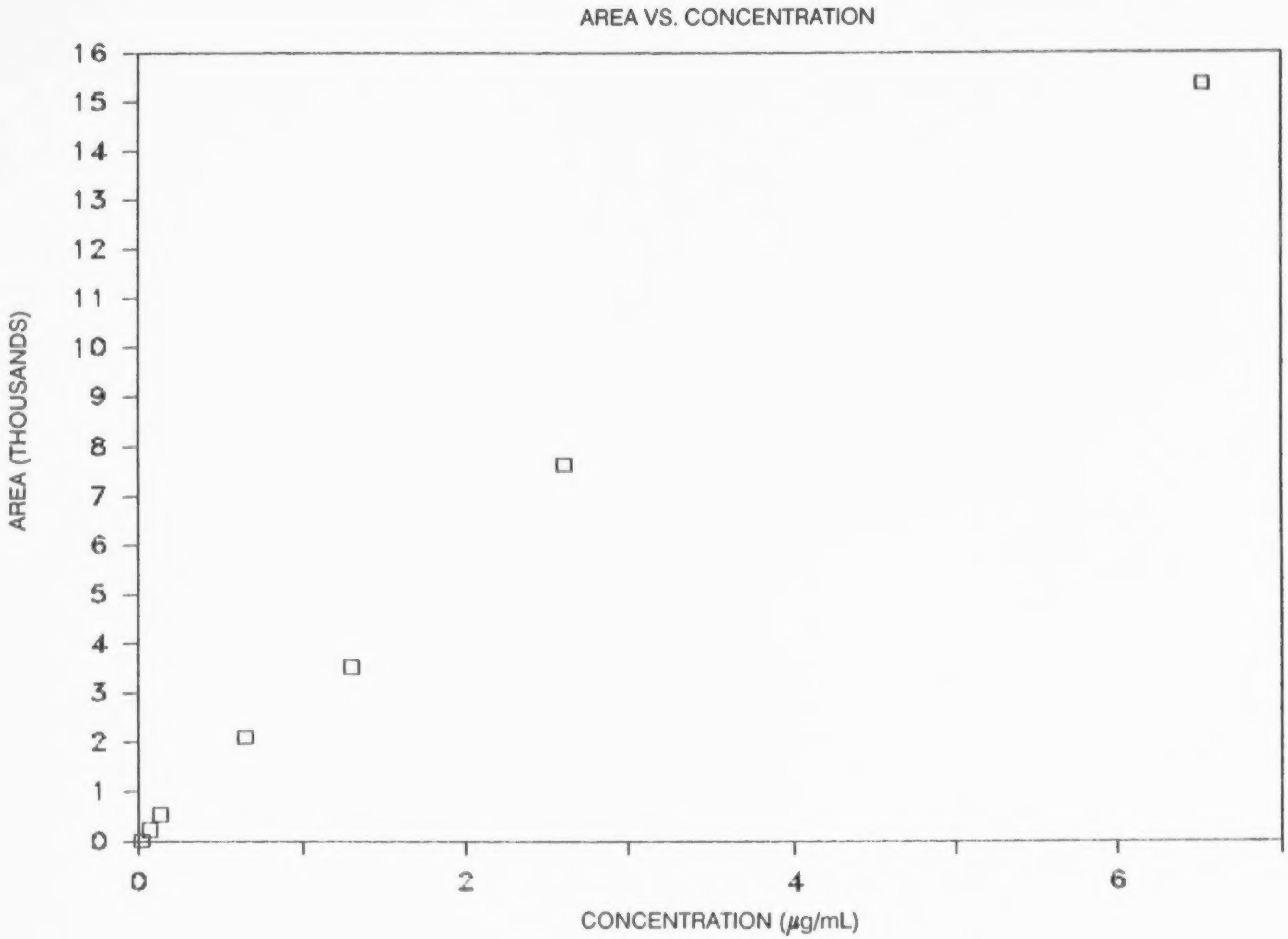


Figure 29B. TEPP: Response (arbitrary units) vs. concentration on the FPD detector.

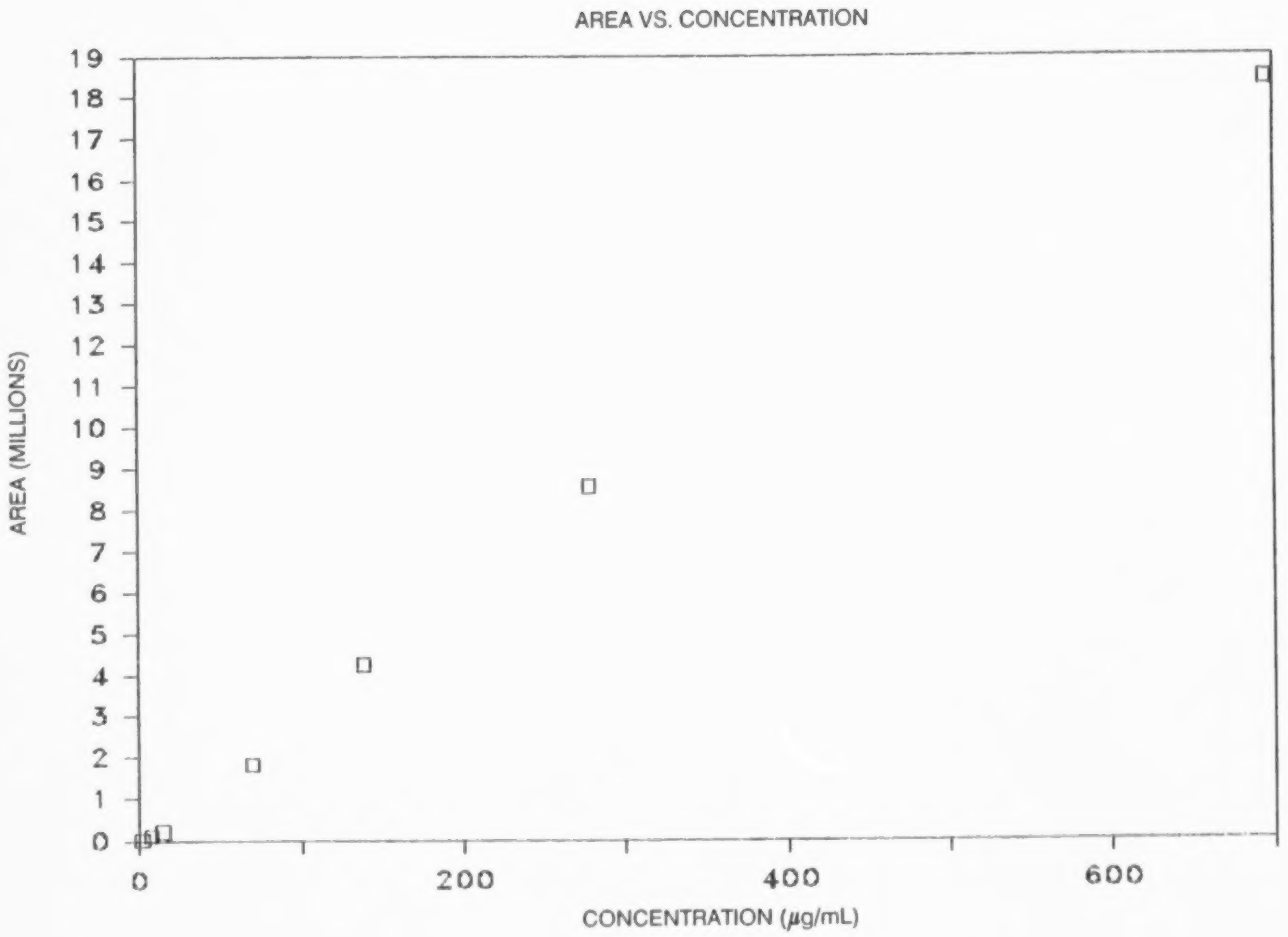


Figure 30A. Tokuthion: Response (arbitrary units) vs. concentration on the NPD detector.

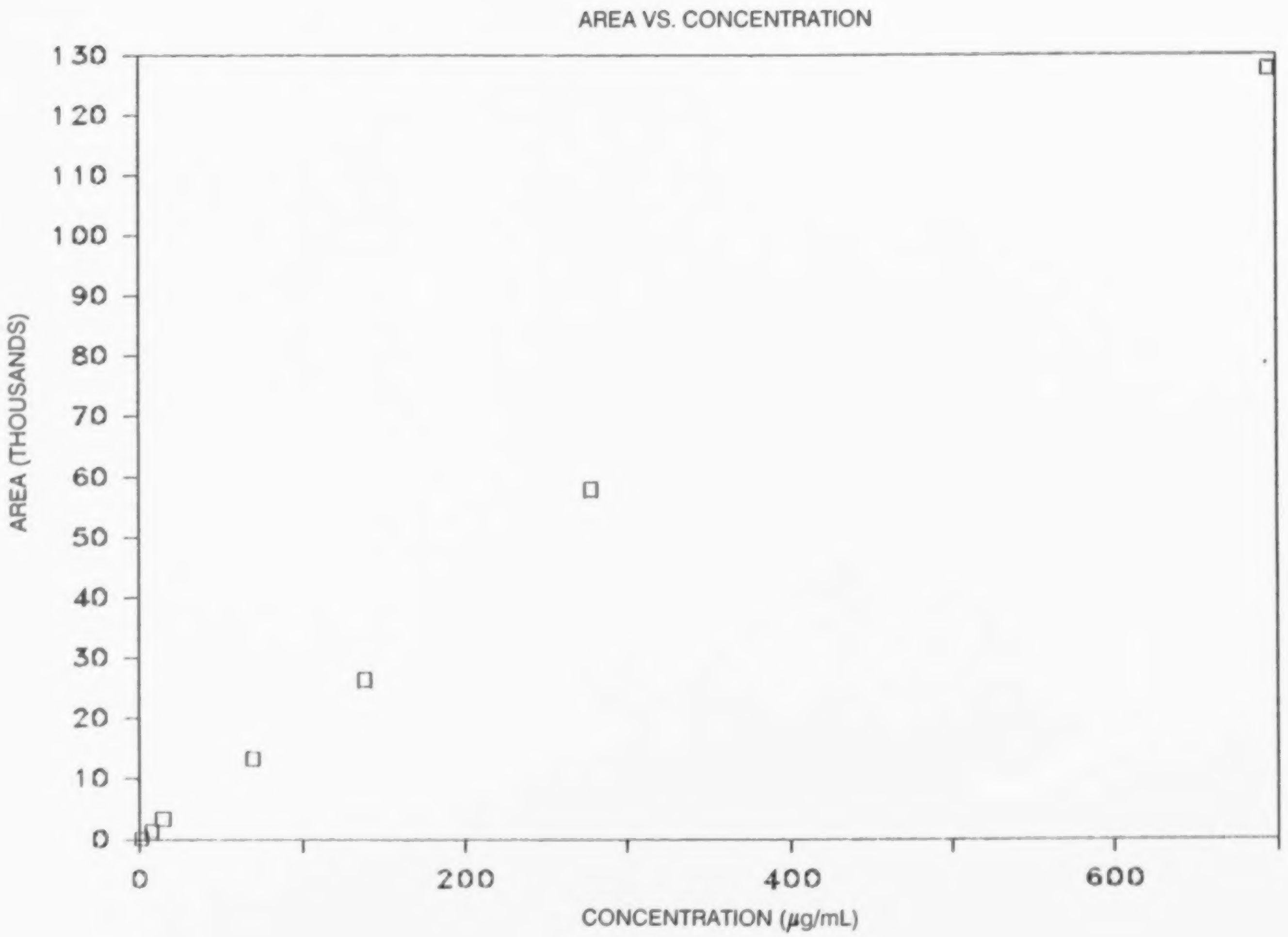


Figure 30B. Tokuthion: Response (arbitrary units) vs. concentration on the FPD detector.

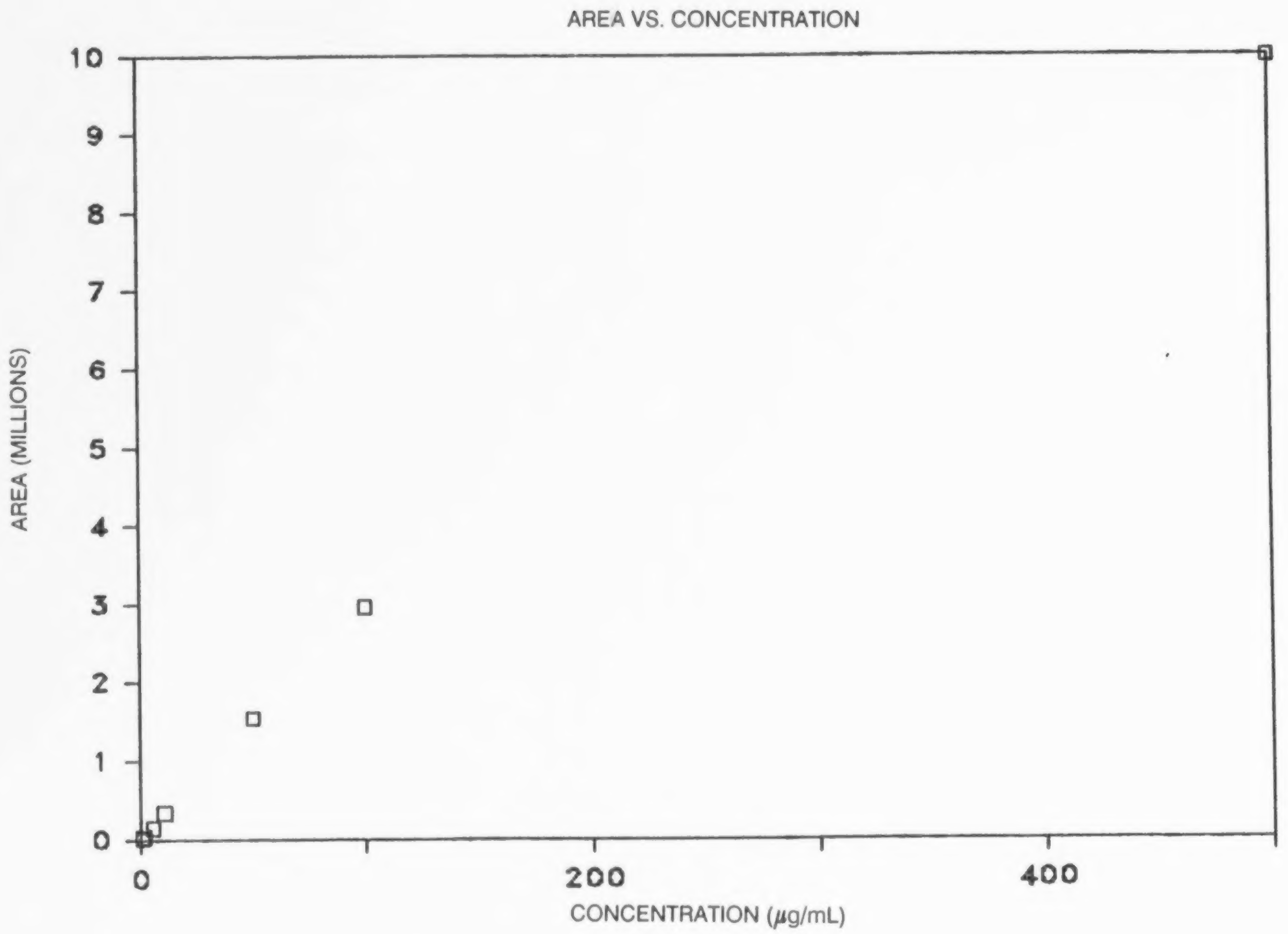


Figure 31. Trichloronate: Response (arbitrary units) vs. concentration on the FPD detector.

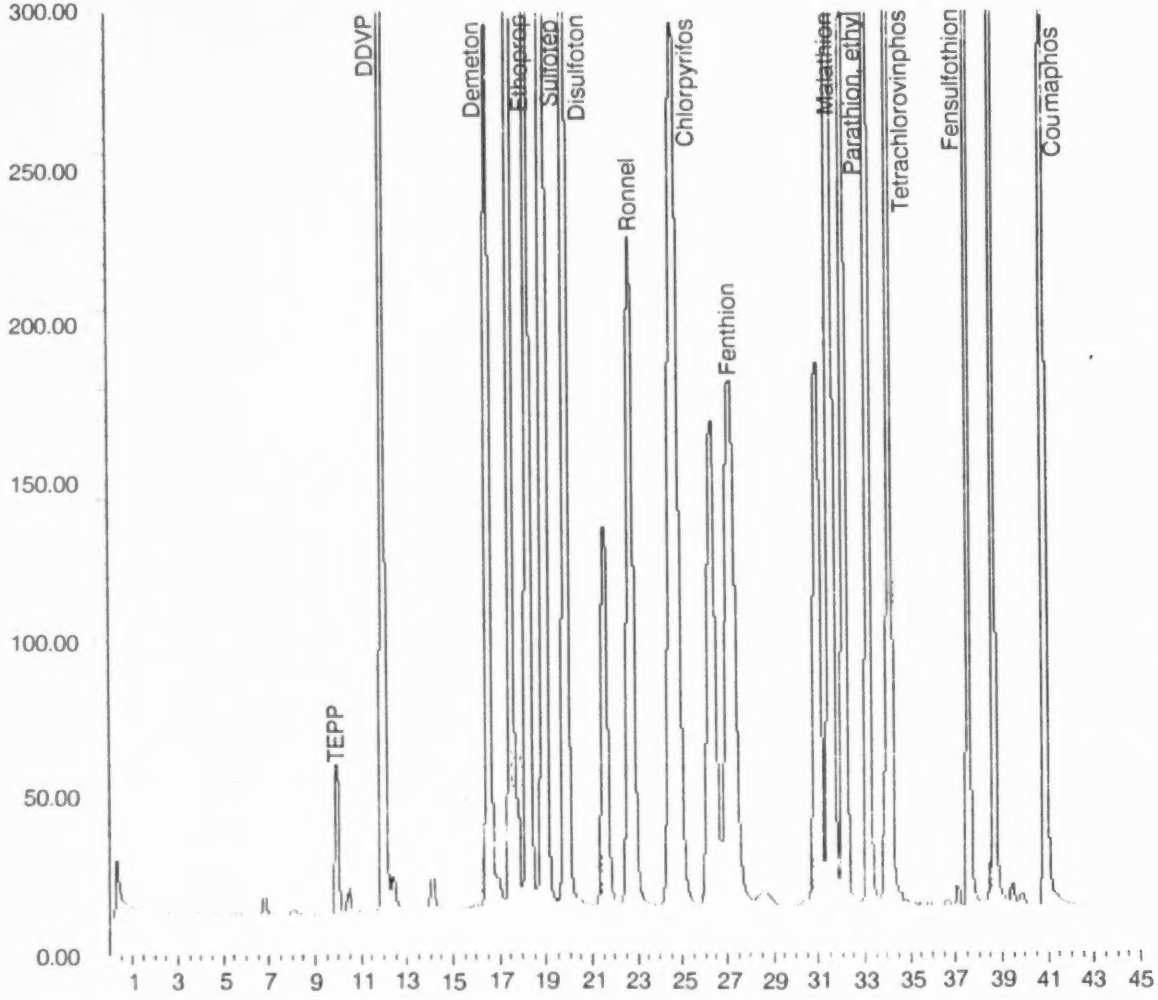


Figure 32. Characteristic response of organophosphates at a FPD detector.

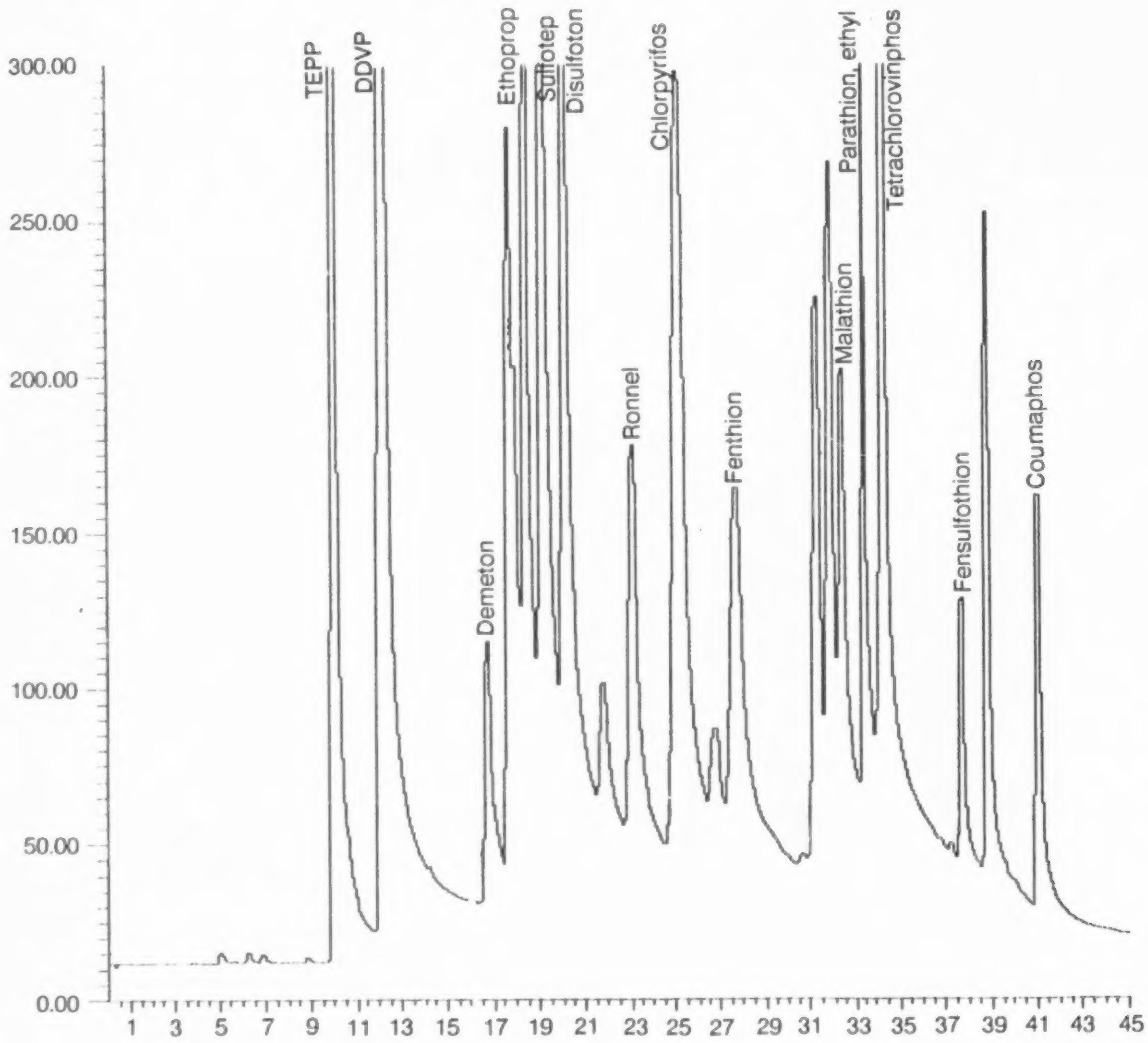


Figure 33. Characteristic response of organophosphates at a NPD detector.

The response factors and detector linear range for the 27 analytes using the NPD are presented in Table 6 and response factors and detector linear range acquired by using the FPD are given in Table 7. The concentrations of the analytes were different and were chosen on the basis of detector response for the particular analyte. The response factors were calculated from the linear portions of the calibration curves (concentration ranges of two to three orders of magnitude) and are reported for each compound. The limit of detection was determined by multiplication of response factor by a peak area ten times the baseline noise.

Detector Recommendations–

Both the NPD and the FPD are suitable for use in Method 8140; the halogen specific detectors currently listed in SW-846 are not suitable because they cannot be used to analyze all of the method analytes. The FPD and the NPD have specific strengths and weaknesses which the analyst should know before selecting a detector.

During detector evaluation, the NPD ceramic bead cracked and rendered the detector unusable. Although this is easy to repair, it confirms the recommendation of Chau[7] that the FPD is the better detector for environmental analysis.

The FPD exhibits an increased sensitivity for compounds containing sulfur because sulfur emissions add to the phosphorous emissions measured at 526 nm.[15] This change in sensitivity is apparent when the response factors for TEPP (2.90×10^2 counts/ng) and Sulfotep (6.97×10^4 counts/ng) are compared. The only difference between these two compounds is that TEPP has two P=0 groups while Sulfotep has two P=S groups (Table 2).

Although detector response was generally linear over the concentration range tested on both detectors, deviation from linearity was observed for the NPD at low concentrations (0.5 to 5 ng on column). This may not be obvious by inspection of the linearity plots (Figures 5-31), but it is obvious in the calculated response factors.

For both the FPD and the NPD, it is necessary to optimize the detector gas flow to give the greatest sensitivity while maintaining detector linearity. It was our experience that the NPD gave good performance when the manufacturers (Hewlett-Packard) recommendations were followed but that FPD sensitivity may be additionally optimized by increasing the hydrogen flow over what was recommended. Regardless of the detector option used or the final flow rates, the analyst must demonstrate detector linearity in the range of concentration to be measured prior to analysis of samples.

TABLE 6. RESPONSE FACTORS FOR 27 METHOD 8140 ANALYTES ON A NITROGEN-PHOSPHOROUS DETECTOR

Compound	Range (ng)	Response Factor (Response/ng)	Instrument Detection Limit (ng)
Azinphos methyl	5-500	2.46×10^4	0.04
Bolstar	0.545-545	6.85×10^3	0.15
Chlorpyrifos	5.2-520	6.27×10^4	0.02
Coumaphos	1.05-525	2.34×10^4	0.04
Demeton	13.7-274	1.09×10^4	0.09
Diazinon	5.8-580	1.68×10^4	0.06
Dichlorvos	4.76-4760	2.05×10^4	0.05
Dimethoate	1.07-107	2.28×10^4	0.04
Disulfoton	0.720-144	5.42×10^5	0.01
EPN	0.62-620	1.61×10^4	0.06
Ethoprop	.61-610	3.81×10^4	0.03
Fensulfothion	6.9-575	3.88×10^4	0.02
Fenthion	7.45-745	5.15×10^4	0.02
Malathion	1.2-600	3.64×10^4	0.03
Merphos	42.6-4260	4.48×10^3	0.22
Mevinphos	7.05-705	2.09×10^4	0.05
Monocrotophos	42.6-4260	2.59×10^3	0.39
Naled	–	ND	ND
Parathion, ethyl	1.35-675	3.69×10^4	0.03
Parathion, methyl	1.28-640	2.74×10^4	0.05
Phorate	1.39-139	1.94×10^4	0.04
Ronnel	0.525-210	3.97×10^5	0.002
Sulfotep	6.67-266	6.78×10^4	0.01
TEPP	6.52-6520	3.71×10^3	0.27
Tetrachlorvinphos	1.03-515	9.17×10^4	0.01
Tokuthion	1.39-695	2.21×10^4	0.04
Trichloronate	–	ND	ND

ND = Not determined.

TABLE 7. RESPONSE FACTORS FOR 27 METHOD 8140 ANALYTES ON A FLAME PHOTOMETRIC DETECTOR

Compound	Range (ng)	Response Factor (Response/ng)	Instrument Detection Limit (ng)
Azinphos methyl	5 - 500	2.28×10^4	0.04
Bolstar	0.545 - 218	5.12×10^4	0.02
Chlorpyrifos	0.52 - 520	6.27×10^4	0.02
Coumaphos	.525 - 525	2.49×10^4	0.04
Demeton	–	ND	ND
Diazinon	1.16 - 232	8.56×10^4	0.01
Dichlorvos	4.76 - 4760	3.08×10^3	0.32
Dimethoate	.535 - 535	2.77×10^4	0.04
Disulfoton	0.720 - 720	2.79×10^4	0.03
EPN	.62 - 248	4.38×10^4	0.02
Ethoprop	0.61 - 610	3.29×10^4	0.03
Fensulfothion	.691 - 690	1.86×10^4	0.05
Fenthion	.745 - 745	2.95×10^4	0.04
Malathion	.60 - 600	2.95×10^4	0.04
Merphos	8.51 - 1700	2.48×10^4	0.04
Mevinphos	14.1 - 705	2.38×10^4	0.04
Monocrotophos	8.57 - 1700	2.47×10^3	0.40
Naled	10 - 500	1.46×10^3	0.68
Parathion, ethyl	.675 - 675	3.53×10^4	0.03
Parathion, methyl	.640 - 640	2.76×10^4	0.04
Phorate	1.39 - 278	3.04×10^4	0.03
Ronnel	0.525 - 525	3.35×10^4	0.03
Sulfotep	0.665 - 266	6.97×10^4	0.01
TEPP	13.0 - 6520	2.90×10^2	3.44
Tetrachlorvinphos	.515 - 515	3.11×10^4	0.03
Tokuthion	.695 - 695	1.96×10^4	0.05
Trichloronate	0.5 - 100	2.98×10^4	0.04

ND = Not determined.

Column Cleanup Procedures

Method 8140 does not include specific cleanup procedures for samples where interferences are a problem. Florisil chromatography is well characterized and has been used successfully for most Method 8140 analytes for many years[16], but the technique is slow. Several workers have applied standard silica gel cleanup methods, but many organophosphates cannot be quantitatively recovered from silica gel microcolumns by standard elution techniques. Recoveries were improved by prewashing water deactivated silica with (20 percent) acetic acid in hexane[17], but this technique is even more time consuming than Florisil. As an alternative to column chromatographic cleanup techniques, rapid vacuum eluted cartridges were used for organophosphorous analysis and procedure is discussed below. GPC was investigated during the soil ruggedness tests and is discussed in Section 5.

Cartridge Cleanup Method--

The apparatus used for the evaluation of cartridge column cleanup was obtained from Analytichem of Harbor City, CA. Equivalent systems are available from Supelco and J.T. Baker. The Bond-Elute system consists of a vacuum manifold which accomodates up to ten 10-mL volumetric flasks as receivers. Sample extracts were cleaned up on propyl diol bonded silica gel (Diol).

Each Diol cartridge is rinsed with hexane, and the rinse is discarded. An aliquot of a multicomponent organophosphorous standard was added to the top of the column to make total analyte on-column approximately 1 µg per component. Three sequential elutions of each column were made as follows:

- Elution 1: 10 mL of 100 percent hexane.
- Elution 2: 10 mL of 10 percent acetone/hexane.
- Elution 3: 10 mL of 20 percent acetone/hexane.

Each fraction was collected and blown down to 1 mL for GC analysis.

Results--

The recovery of eight organophosphorous compounds from Diol cartridge columns was determined and are reported in Table 8. The results are excellent for seven of the eight compounds. Sulfotep was not recovered in any of the three elutions, nor is it recovered from Florisil columns.[16] Malathion was quantitatively recovered in the second elution (10 percent acetone/hexane); this demonstrated that the elution patterns of phosphates on Diol and florisil are similar. None of the organophosphorous compounds of this group were recovered in the third elution. The eluates may either be combined for analysis or a single elution using 10 percent acetone/hexane if the chromatographic system will provide sufficient resolution.

TABLE 8. RECOVERY OF EIGHT ORGANOPHOSPHOROUS INSECTICIDES FROM DIOL CARTRIDGE COLUMN

Compound	100% Hexane	10% Acetone/Hexane	20% Acetone/Hexane
Chlorpyrifos	100	–	–
Demeton	123	–	–
Disulfoton	95	–	–
Fenthion	101	–	–
Malathion	–	116	–
Parathion, ethyl	100	–	–
Phorate	95	–	–
Sulfotep	NR	NR	NR

NR = Not recovered.

Recommendations–

When Method 8140 is applied to complex environmental samples, it may be necessary to remove polar materials that degrade chromatography or that cause the breakdown of labile organophosphates. Although no cleanup method has been validated for use as part of this method, three column techniques are suggested as options: acid-washed silica, Florisil, and Diol cartridge columns.

Acid washed silica gel[17] or Florisil[16] cleanup techniques may be applied for the analysis of most Method 8140 analytes if appropriate cautions are employed. Cartridge cleanup by using polar Diol columns may be also applied to extracts using the elution solvents presented here. Diol cartridges offer distinct advantages over conventional column cleanup: (1) less time is required to prepare samples, and (2) interlaboratory repeatability is improved because columns are prepacked by the manufacturer with the identical dimensions and the same amount of packing. If any cleanup techniques are applied to samples analyzed by Method 8140, the laboratory must determine analyte recoveries by using the particular lot of adsorbent and the organophosphorous standards to be determined prior to the analysis of samples.

METHOD OPTIMIZATION

The sample preparation steps for water and soil samples were optimized for use in Method 8140. The results of the optimization study are reported in this section. The optimized soil procedure was then applied to hazardous waste samples to demonstrate that the method was valid for the analysis of wastes. Also reported here are analytical difficulties with specific organophosphorous analytes that were observed in this study.

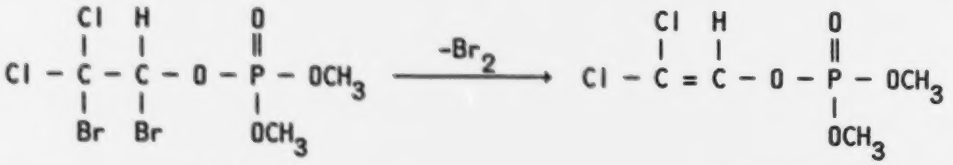
Analytical Difficulties Encountered with Specific Organophosphorous Pesticides

The measurement of several of the compounds listed in Table 1 present special difficulties to the analyst using Method 8140.

TEPP gave variable and generally lower recoveries than other compounds studied in part because of its volatility. In addition, TEPP is a relatively unstable diphosphate which is rapidly hydrolized in water[18] and which is thermally labile (TEPP decomposes at 170°C). This instability makes losses in the GC analysis or during sample preparation inevitable. As the major breakdown product of TEPP is triethylphosphate[19] which gives an EI mass spectrum very similar to that of the parent compound[20], identification of bad lots of the standard is difficult.

Recoveries of dichlorvos are low and variable because it is volatile and because it has a relatively high aqueous solubility, 10 g/L at 20°C.[4] This water solubility results in poor extraction efficiency from samples.

Naled is converted to dichlorvos on column by the debromination reaction presented here.[21]



$$\mathrm{Cl}-\underset{\mathrm{Br}}{\overset{\mathrm{Cl}}{\mathrm{C}}}-\underset{\mathrm{Br}}{\overset{\mathrm{H}}{\mathrm{C}}}-\mathrm{O}-\underset{\mathrm{OCH_3}}{\overset{\mathrm{O}}{\overset{\|}{\mathrm{P}}}}-\mathrm{OCH_3}\xrightarrow{-\mathrm{Br_2}}\mathrm{Cl}-\overset{\mathrm{Cl}}{\mathrm{C}}=\overset{\mathrm{H}}{\mathrm{C}}-\mathrm{O}-\underset{\mathrm{OCH_3}}{\overset{\mathrm{O}}{\overset{\|}{\mathrm{P}}}}-\mathrm{OCH_3}$$

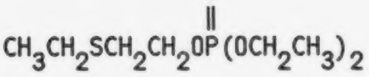
Naled Dichlorvos

This reaction may also occur also during sample work-up. The extent of the debromination will depend on the nature of the matrix being analyzed. The potential for debromination must be considered when Naled is to be determined by Method 8140.

Trichlorfon rearranges and is dehydrochlorinated in acidic, neutral, or basic media to form dichlorvos and hydrochloric acid.[5] If this method is to be used for the determination of organophosphates in the presence of trichlorfon, the analyst should be aware of the possibility of rearrangement to dichlorvous in order to avoid misidentification.

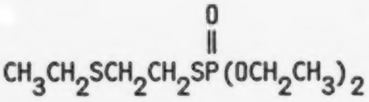
Demeton is a mixture of two isomers, a thiophosphate (Demeton-O) and its isomereic oxon (Dementon-S).

Demeton-O



$$CH_3CH_2SCH_2CH_2O\overset{\overset{S}{\|}}{P}(OCH_2CH_3)_2$$

Demeton-S



$$CH_3CH_2SCH_2CH_2S\overset{\overset{O}{\|}}{P}(OCH_2CH_3)_2$$

The isomerization of any thiophosphate to the corresponding oxone is spontaneous, but the rate of conversion is a function of storage conditions and of the amount of oxygen dissolved in the sample.[22] Two peaks were observed in all Demeton standards obtained; however, only the retention time for the oxone is reported. This assignment of retention times can be made based on previous studies which have shown that oxons elute earlier than the corresponding thions.[23] Although recovery studies on each of the isomers have been reported elsewhere, separate standards for Demeton-O and Demeton-S are no longer available through EPA. Therefore, all recovery studies were performed with standards containing both isomers.

Mevinphos is a mixture of E and Z isomers. Only the retention time of the active E isomer[24] is reported. As mevinphos is produced as a mixture with varying amounts of each isomer, response factors based on the E isomer only may not be reproducible between different lots of mevinphos standard.

Merphos, tributyl phosphorotrithioite is a single component pesticide that is readily oxidized in the environment and during storage by the reaction given below:[25]

$$(n\text{-}C_4H_9S)_3\text{-}P \xrightarrow{[O]} (n\text{-}C_4H_9S)_3P = O$$

In this study two peaks were observed for merphos in all analyses, but no standard for the oxidation product was available to determine its response factor. Therefore, quantitation was based on the unoxidized merphos. This approach will be successful only if the same fraction of merphos in the sample and of merphos in the standard is oxidized during sample preparation and analysis.

Methyl parathion is a thermally labile and chemically reactive compound that decomposes during sample preparation and analysis.[26] The recoveries of methyl parathion are significantly lower than ethyl parathion.

The chromatogram of the multicomponent standards and samples used the temperature programs given in Table 5 and showed distinct peak shape variations which were dependent on where analytes elute in the chromatogram. It was found that the peaks which elute in the central, isothermal portions of the chromatograms, such as malathion, methyl parathion, and tokuthion, were broadened in relation to the peaks in either the earlier or later portions of the chromatogram. This affected the quantitation of these analytes because it is more difficult to detect broadened peaks than the sharp peaks obtained during the temperature program. The isothermal period was required in the programs because some compounds could not be separated when a temperature ramp was used during the entire run.

Optimization of Method 8140 for the Preparation and Analysis of Aqueous Samples

Two alternative extraction techniques for water are allowed in Method 8140. The methods were optimized for water and were compared as to accuracy and precision by using water spiked at several concentrations and extracted according to the procedures described below.

Method 3510: Separatory Funnel Extraction–

A one-liter sample of distilled water was placed in a two-liter separatory funnel. An appropriate aliquot of a multicomponent organophosphorous standard in acetone was added. The water sample was extracted with three 60-mL portions of methylene chloride, and the extracts were combined. The extract is transferred to a K-D evaporator, is concentrated to less than 10 mL, and is exchanged to hexane on a steam bath. The concentrated extract was transferred to a vial and was concentrated to 1 mL under a stream of dry nitrogen. The 1-mL extract was transferred to an autosampler vial analyzed by GC.

Method 3520: Continuous Liquid-Liquid Extraction–

A one-liter sample of distilled water was placed in a two-liter continuous extractor. An appropriate aliquot of a multicomponent organophosphorous standard in acetone was added. Sufficient methylene chloride was added to the receiving flask and to the extractor to ensure continuous cycling. The receiving flask was heated, and the sample was extracted for 16 hours. The extract was concentrated and prepared for analysis in an identical manner as separatory funnel extractions.

Recovery of Selected Compounds–

Recovery of a mixture of selected organophosphorous analytes from water by Methods 3510 and 3520 over a concentration range from 1 µg/L to 100 µg/L was determined; the results are summarized in Table 9. Recoveries are reported as the

TABLE 9. PERCENT RECOVERIES BY METHOD 3510 AND METHOD 3520

Compound	Spiked Concentration	Method 3510 Percent Recovery	Method 3520
Chlorpyrifos	Low	69 ± 5	50 ± 8
	Medium	101 ± 6	109 ± 12
	High	69 ± 4	ND
DDVP	Low	ND	23 ± 5
	Medium	28 (n=2)	ND
	High	75 ± 12	ND
Disulfoton	Low	61 ± 10	32 ± 8
	Medium	84 ± 9	71 ± 8
	High	77 ± 5	ND
Fenthion	Low	54 ± 16	37 ± 9
	Medium	105 ± 8	118 ± 11
Malathion	Low	54 ± 17	50 ± 13
	Medium	112 ± 9	162 ± 14
	High	116 ± 8	ND
Parathion, ethyl	Low	87 (n=2)	LMQ
	Medium	134 ± 11	114 ± 12
	High	78 ± 4	ND
Phorate	Low	51 ± 15	55 ± 25
	Medium	84 ± 9	87 ± 6
	High	67 ± 4	ND
Sulfotep	Low	63 ± 14	54 ± 1
	Medium	95 ± 12	101 ± 9
	High	70 ± 5	ND

Recoveries and standard deviations are reported for n = 10 replicate determination except where noted.

Low = 1 μg/L
Medium = 10 μg/L
High = 100 μg/L

LMQ = Less than minimum quantitatible.
ND = Not determined.

mean and standard deviation calculated from the results of ten replicate extractions except where noted.

All extractions were done with methylene chloride as a solvent. Although previous studies have demonstrated that extraction with chloroform[27] or benzene[28] gives better recovery for some compounds than does methylene chloride, neither of these solvents was used in this study because of the chronic toxicity/carcinogenicity of benzene and chloroform.

Although the solvent mixture of 15 percent methylene chloride in hexane is specified in EPA Method 614, it was not tested because it has been demonstrated previously to be a less effective than methylene chloride in extracting organophosphates from water.[29] Although some authors have reported that it is possible to "salt out" phosphate pesticides from water[30] samples in order to increase recoveries, studies in this laboratory showed no difference in recoveries of phosphates after the addition of ammonium acetate when they were compared with regular methylene chloride extraction.

Methylene chloride extracts were analyzed by using two packed GC columns (DEGS or 6% OV-210/4% SP-2100) and the FPD. Peak broadening, especially on the DEGS column, made quantitation of most analytes spiked at low concentrations difficult. The DEGS column was used in order to quantitate DDVP on packed column. The observed recoveries were generally in the 50 to 100 percent range except for dichlorvos, ethyl parathion, and malathion. Dichlorvos gave poor recoveries both because it has the highest water solubility of the analytes tested and because it elutes close to the solvent peak. Ethyl parathion and malathion gave high recovery values (112-162 percent) which were presumably due to interference problems on the packed column (Figure 34). Hydrolysis of analytes during overnight extraction may be responsible for the slightly lower recoveries obtained with continuous extraction (Method 3520) versus separatory funnel shakeout (Method 3510). This difference in recovery was also observed in the validation study reported in Section 6 and has been reported previously by Zweig and Devine when hexane is used as an extraction solvent for phosphate pesticides.[31]

The water recovery study was repeated, and the recoveries for 17 selected analytes spiked into clean water at a concentration of 1.5 µg/L are reported in Table 10. These recoveries were determined by using separatory funnel extraction and megabore capillary (DB-210) GC analysis. Trichloronate was not recovered, but the other 16 compounds gave good results (56-98 percent). The compounds that gave recoveries of over 100 percent on packed column (ethyl parathion, and malathion) gave better results with capillary column analysis; this indicates that the analytical problems were

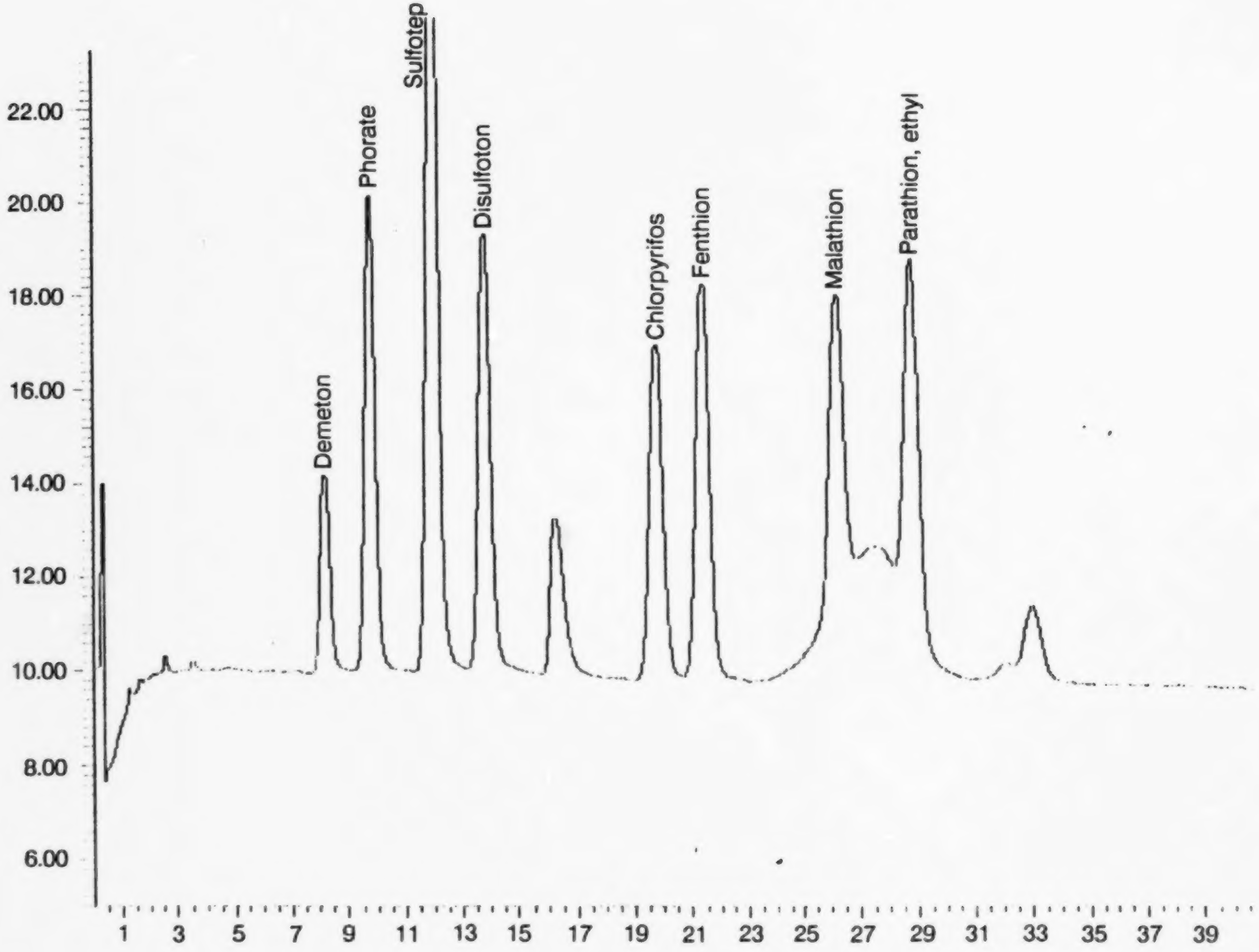


Figure 34. Interference observed in packed column analysis of a water extract.

caused by poor chromatography. Even with capillary analysis, dichlorvos, dimethoate, and fensulfothion gave lower than 70 percent recoveries and relatively large standard deviations. The low recoveries and high variability for these three compounds are presumably a result of their high aqueous solubilities which are three orders of magnitude higher than all the other Method 8140 parameters (Table 3). TEPP also gave poor recovery, presumably because this compound is both highly volatile and subject to hydrolytic and thermal degradation.

TABLE 10. AVERAGE RECOVERY OF 17 ORGANOPHOSPHOROUS INSECTICIDES (AT ~1.5 μg/L) FROM WATER BY METHOD 3510 USING CAPILLARY COLUMN ANALYSIS (DB210)

Compound	Average Recovery (5 Replicates)	Percent RSD
Azinphos methyl	83 ± 9	11
Coumaphos	76 ± 6	8
DDVP	62 ± 10	16
Demeton oxone	56 ± 11	20
Dimethoate	65 ± 25	38
Disulfoton	76 ± 12	16
Fensulfothion	66 ± 22	34
Fenthion	81 ± 11	13
Malathion	80 ± 6	7
Parathion, ethyl	80 ± 7	9
Parathion, methyl	84 ± 8	10
Phorate	91 ± 12	13
Ronnel	80 ± 6	7
Sulfotep	81 ± 8	10
TEPP	27 ± 6	22
Tetrachlorvinphos	98 ± 17	17
Trichloroanate	LMQ	

LMQ = Less than minimum quantitatable.

A sample chromatogram for the packed column analysis is presented in Figure 34. The interference observed in the region of malathion and parathion that resulted in unacceptably high recovery can be seen in this figure. A sample chromatogram for the water extracts on the DB-210 capillary column is presented in Figure 35; it is interference free. As can be seen from these two figures, megabore capillary column analysis is a significant improvement over packed column analysis both in its resolution of analytes and in reduction of chromatographic interferences.

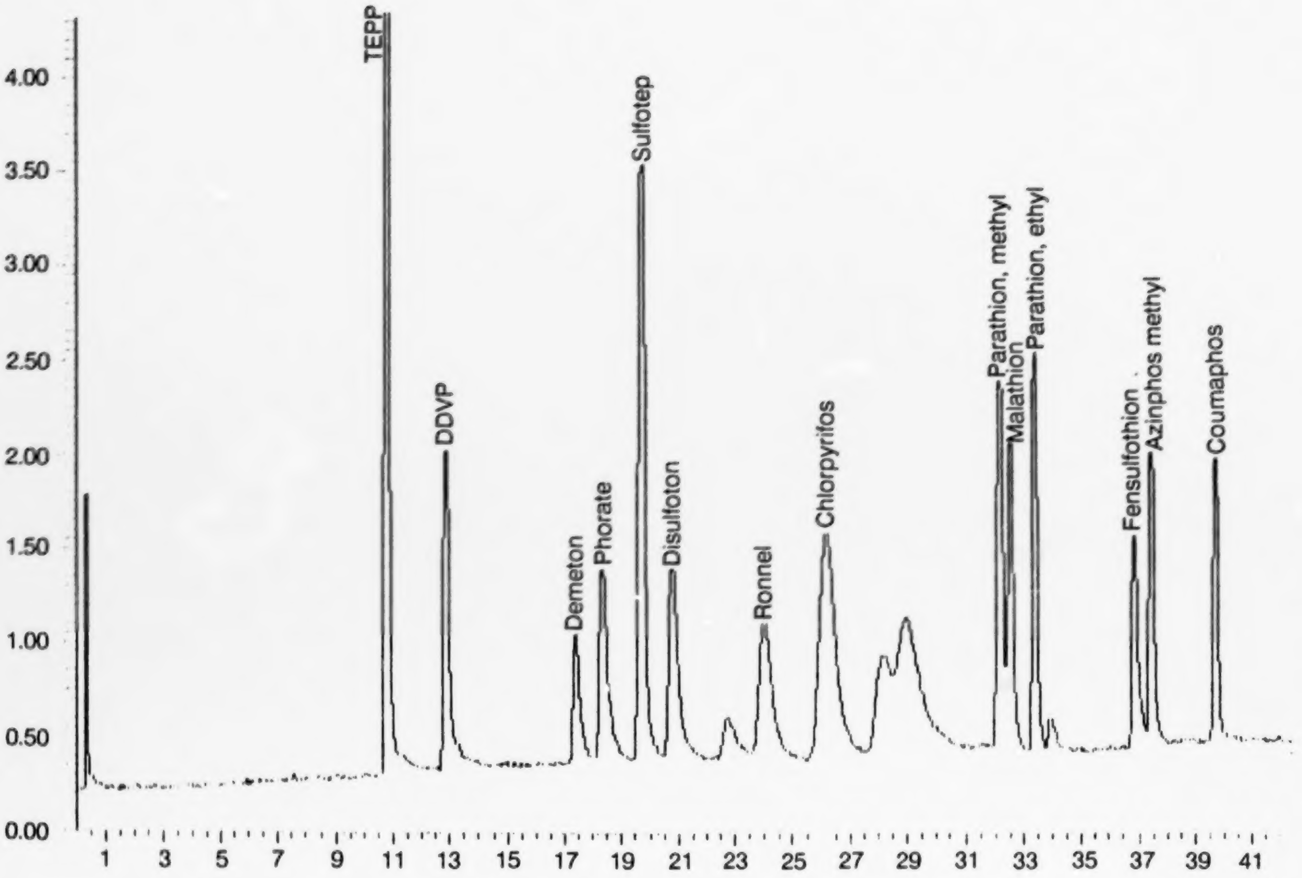


Figure 35. Water extract chromatogram on a DB-210 megabore capillary column.

Recommendations for Aqueous Sample Extraction--

The two methods of aqueous sample preparation, separatory funnel extraction (Method 3510) and continuous liquid-liquid extraction (Method 3520), are both acceptable methods for extraction of organophosphates from aqueous samples. While there is some evidence from the results reported here as well as from previously published report[31] that the separatory funnel gives better recoveries than does the continuous liquid extractor, the difference is small. Because of potential loss of analyte during separatory extraction of environmental samples which is due to the formation of emulsions, no recommendation of separatory funnel shakeout over continuous liquid extraction for sample preparation of aqueous samples is made for Method 8140.

Optimization of Method 8140 for the Preparation and Analysis of Soil Samples

A spiking solution containing 13 organophosphorous parameters was prepared in acetone in order to determine recovery from soils. Two methods of sample extraction are specified for soils and sediments in Method 8140: soxhlet extraction (Method 3540) and sonication (Method 3550). These methods were compared as to method accuracy and precision by the procedures summarized below.

Soxhlet Extraction--

Ten grams of soil were weighed to the nearest 0.1 g and were placed in a cellulose extraction thimble. A 0.150-mL aliquot of the multicomponent organophosphorous standard in acetone was added and was mixed thoroughly with the soil. The soxhlet extraction apparatus was assembled, and sufficient 1:1 methylene chloride/acetone was added to the round bottom flask to ensure continuous cycling. The extraction was stopped after 16 hours, and the solvent transferred to a K-D flask, exchanged to hexane, and concentrated to a volume less than 10 mL. The extract was then transferred to a three-dram vial and concentrated under a stream of nitrogen to a final volume of 1 mL prior to analysis.

Extraction by Sonication--

Thirty grams of soil were weighed to the nearest 0.1 g into a 250-mL beaker. A 0.150-mL aliquot of a multicomponent organophosphorous standard in acetone was added and was thoroughly mixed with the soil. Approximately 60 g of anhydrous sodium sulfate was added and mixed with the soil. The soil was extracted with three 80-mL portions of 1:1 acetone/methylene chloride by using a 375 watt sonicator which was set with the operating power at 30 to 40 percent output power. A 1/2- or 3/4-inch horn was used, and placed 1 cm below the level of the solvent. Each extract was filtered through sodium sulfate, and the three portions were combined in a 500-mL round bottom flask. The remaining soil was quantitatively transferred to the filter and was rinsed with 60 mL of clean methylene chloride, and the rinse was added to the combined extracts. The extract was solvent exchanged to hexane and was concentrated in an identical manner as for the soxhlet extraction.

Recovery of Selected Compound from Soil--

The recoveries and percent relative standard deviations for 13 analytes acquired by using both sonication and soxhlet extraction are reported in Table 11. The compounds were selected to represent the range of physical and chemical properties of the 27 method parameters tested. The GC analysis was accomplished with a SPB-608 capillary column. The recoveries range between 93 percent and 122 percent for Method 3540 (with one outlier) and between 81 percent and 106 percent for Method 3550 (with one outlier).

TABLE 11. AVERAGE RECOVERY OF 13 ORGANOPHOSPHOROUS INSECTICIDESFROM SOIL SPIKED AT 50 μg/kg BY METHOD 3540 AND METHOD 3550

Compound	Recovery Method 3540 Soxhlet Extraction	Percent RSD	Recovery Method 3550 Sonication	Percent RSD
Azinphos methyl	111 ± 7	6	89 ± 10	11
Chlorpyrifos	103 ± 6	6	90 ± 10	11
Coumaphos	186 ± 8	4	92 ± 11	11
DDVP	98 ± 20	20	177 ± 50	28
Disulfoton	93 ± 5	5	81 ± 6	7
EPN	121 ± 9	7	105 ± 7	7
Fenthion	104 ± 6	6	88 ± 8	9
Malathion	110 ± 5	4	106 ± 11	10
Parathion	94 ± 11	11	105 ± 9	8
Phorate	122 ± 5	4	96 ± 7	7
Ronnel	96 ± 5	5	91 ± 6	6
Sulfotep	98 ± 5	5	94 ± 6	6
Tetrachlorvinphos	121 ± 15	12	97 ± 13	13

Analyses completed by using DB-608 column, ten replicate determinations for each column-analyte combination.

All values are reported as the mean and standard deviation for 10 replicate extractions, except where noted. A high recovery (186 percent) was reported for coumaphos when use was made of the soxhlet extraction procedure. Examination of the sample chromatograms showed that there was a coelution problem from late eluting interferences in the soxhlet chromatograms. Examination of the recovery data shows that the soxhlet recoveries of all of the later eluting analytes were greater than 100 percent because of the elevated baseline. This late eluting interference was not observed in the samples prepared by sonication, and this indicates that some matrix materials are extracted more efficiently by soxhlet extraction than by sonication. The high dichlorvos recovery (177 percent) was caused by a more volatile interferent that was extracted by the sonicator but that was not extracted by the soxhlet. Soxhlet extraction requires heating of the sample which may have volatilized some early eluting interference. Thus, soxhlet samples had an interference that eluted late in the chromatographic run while the sonicator samples had an interference that eluted early.

The recovery for the 13 compounds from soil subsequently was determined by using sonication only at three concentration levels (5 µg/kg, 50 µg/kg and 500 µg/kg). The results are reported in Table 12 and a representative chromatogram of soil spiked at the high level is presented in Figure 36. The recoveries are comparable with those reported in Table 11 and are concentration independent. The only outlier was dichlorvos which was not detected at low concentration and which was recovered at very high levels at the medium and high concentrations. These variable recoveries demonstrate that dichlorvos is a difficult compound to analyze.

Recommendations for Solid Sample Extraction--

Both methods, soxhlet (Method 3540) and sonication (Method 3550), are acceptable techniques for the extraction of solid samples in Method 8140. The analyst should be aware that particular samples may be more amenable either to soxhlet or to sonication.

Application of Method 8140 to the Preparation and Analysis of Hazardous Waste Samples

Drilling mud samples supplied by the Industrial Technology Division (ITD) of the EPA were spiked with TEPP plus the 13 analytes used for the soil recovery studies and were then extracted by sonication using a 1:1 methylene chloride/acetone mixture according the extraction procedure described for soil samples. The extracts were concentrated and analyzed by GC using a DB-210 column and an FPD.

Recovery of Selected Organophosphorous Compounds from Hazardous Waste Samples

A hazardous waste sample was spiked at three concentrations, (5 µg/kg, 50 µg/kg, and 500 µg/kg), and ten replicate extracts were analyzed at each concentration

TABLE 12. AVERAGE RECOVERY OF 13 ORGANOPHOSPHOROUS INSECTICIDES FROM SOIL SPIKED AT THREE ANALYTE CONCENTRATIONS BY METHOD 3550

Concentration Compound	5 µg/kg	Percent RSD	50 µg/kg	Percent RSD	500 µg/kg	Percent RSD
Azinphos methyl	95 ± 7	7	89 ± 10	11	74 ± 13	18
Chlorpyrifos	73 ± 16	22	90 ± 10	11	80 ± 5	6
Coumaphos	86 ± 8	9	92 ± 11	12	85 ± 7	8
DDVP	ND	--	117 ± 50[c]	28	214 ± 24	11
Disulfoton	89 ± 11	13	81 ± 6	7	87 ± 4	5
EPN	93 ± 7	8	105 ± 7	7	100 ± 5	5
Fenthion	109[a]	--	88 ± 8	9	83 ± 5	6
Malathion	103[a]	--	106 ± 11	10	100 ± 6	6
Parathion	97[a]	--	105 ± 9	9	100 ± 6	6
Phorate	95 ± 8	9	96 ± 7	8	88 ± 4	5
Ronnel	86 ± 9	11	91 ± 6	6	90 ± 5	5
Sulfotep	90 ± 7	8	94 ± 6	6	89 ± 7	8
Tetrachlorvinphos	114 ± 14[b]	13	97 ± 13	14	94 ± 6	7

All analyses performed on SPB 608. The mean standard and RSD are reported for ten replicates except where noted.

[a] Quantitated in one samples.
[b] Average and standard deviation of seven replicates.
[c] Average and standard deviation of nine replicates.

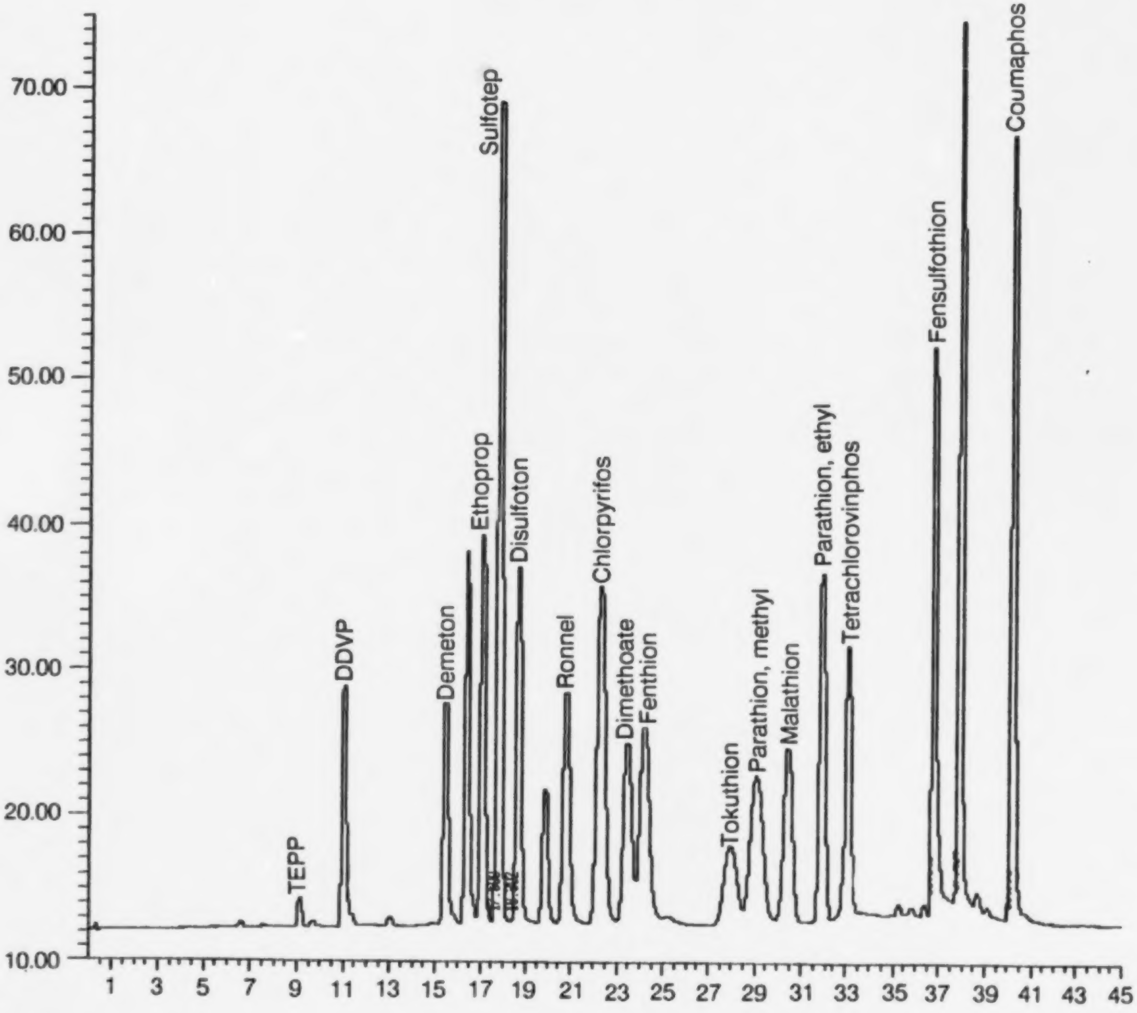


Figure 36. Soil extract chromatogram on a DB-210 megabore capillary column with FPD detection.

level. The hazardous waste sample chosen for the recovery experiments was sent to S-CUBED for determination of organochlorine insecticides as part of another EPA project. This particular sample was selected because it had a large number of fine particles and because it presented difficulties during previous extractions. It was felt that this sample was a difficult test for demonstrating the suitability of Method 8140 for environmental samples. The analysis of the unspiked sample showed the presence of an unidentified peak that eluted in the region of mevinphos, phorate, and ethoprop on the DB-210 column. A representative sample chromatogram of hazardous waste spiked at medium concentration is presented in Figure 37.

Analyte recoveries are reported in Table 13. The recovery of most of the compounds was between 50 and 90 percent at all three concentrations. Only malathion, methyl parathion, and TEPP were not recovered in the lowest concentration spikes. At the medium and high concentrations, recoveries were >50 percent with the exception of TEPP at medium concentration. The relative standard deviation (RSD) was <20 percent at high concentration for all 13 compounds. The RSD's were higher at low concentration but in no case were they greater than 32 percent. This study was a good demonstration of the validity of modified Method 8140 for the analysis of organophosphorous analytes in hazardous waste.

TABLE 13. RECOVERY OF 14 ORGANOPHOSPHOROUS FROM HAZARDOUS WASTE BY METHOD 3550

Compound	Low	Percent RSD	Medium	Percent RSD	High	Percent RSD
Azinphos methyl	78 ± 14	18	77 ± 9	11	71 ± 10	14
Chlorpyrifos	79 ± 23	29	77 ± 10	13	75 ± 7	9
Coumaphos	75 ± 17	23	89 ± 11	12	77 ± 11	14
Demeton	71 ± 21	29	60 ± 8	14	56 ± 5	9
Dichlorvos	85 ± 13	15	71 ± 13	18	107 ± 16	15
Dimethoate	52 ± 13	26	62 ± 10	16	51 ± 9	18
Disulfoton	98 ± 29	29	73 ± 4	5	64 ± 6	9
Fensulfothion	86 ± 10	11	76 ± 11	15	72 ± 10	14
Fenthion	88 ± 28	32	82 ± 14	17	62 ± 8	13
Malathion	LMQ	--	57 ± 6	10	53 ± 5	9
Parathion, ethyl	64 ± 18	28	61 ± 8	13	54 ± 5	9
Parathion, methyl	LMQ	--	83 ± 10	12	59 ± 6	10
Sulfotep	74 ± 13	17	57 ± 4	7	57 ± 4	7
TEPP	LMQ	--	--	--	95	11

All analyses performed on DB-210. Ten replicate determinations performed at each concentration.

LMQ = Less than minimum quantitable.

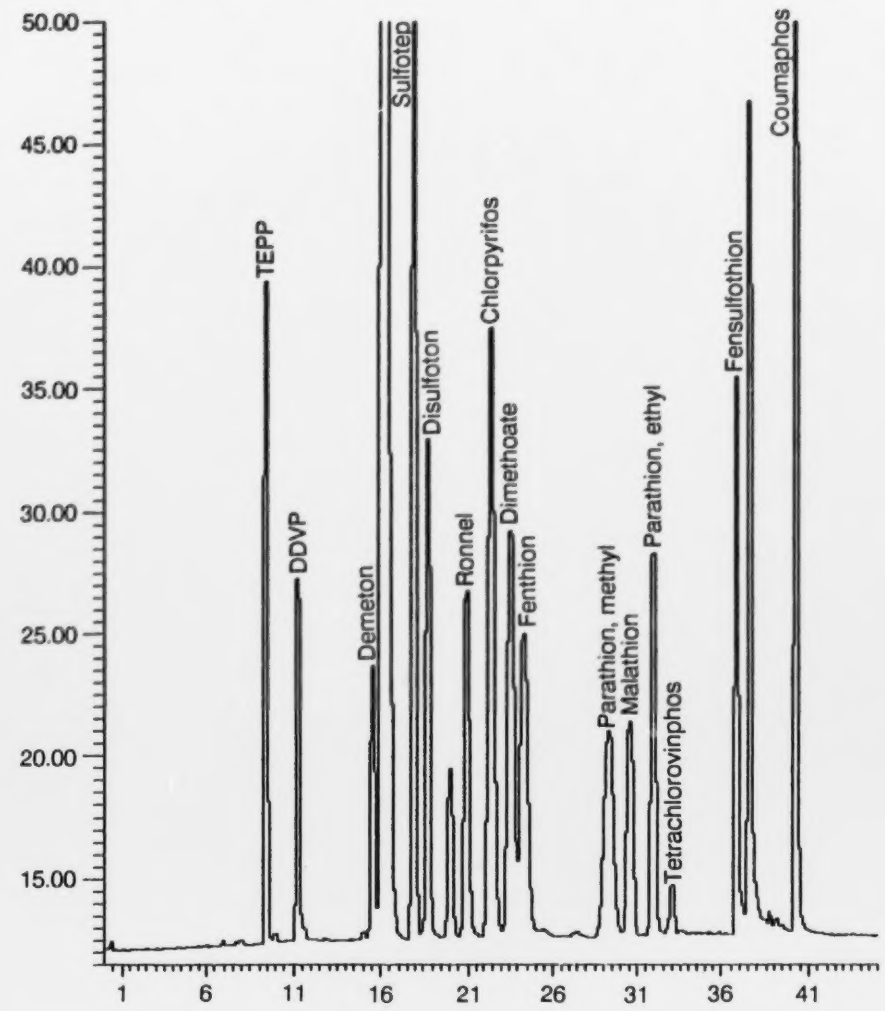


Figure 37. Hazardous waste extract chromatogram on a DB-210 megabore capillary column with FPD detection.

RUGGEDNESS TESTING

The ruggedness test design of W.J. Youden[3] was used to determine the level of significance for seven method variables in eight experiments. Two levels of each experimental condition are assigned capital or lower case letters and are varied in each of the eight determinations according to the matrix pictured in Table 14.

TABLE 14. COMBINATIONS OF SEVEN FACTORS USED TO TEST THE RUGGEDNESS OF AN ANALYTICAL METHOD

Factor Value	Combination or Determination No.							
	1	2	3	4	5	6	7	8
A or a	A	A	A	A	a	a	a	a
B or b	B	B	b	b	B	B	b	b
C or c	C	c	C	c	C	c	C	c
D or d	D	D	d	d	d	d	D	D
E or e	E	e	E	e	e	E	e	E
F or f	F	f	f	F	F	f	f	F
G or g	G	g	g	G	g	G	G	g
Observed result	s	t	u	v	w	x	y	z

The differences in the experimental result obtained by changing a single condition are determined by taking the average of the results for the determination assigned a capital letter minus those assigned the lower case. For example, changing a condition designated by a capital "F" to that condition designated by the lower case "f", the difference (D_f) is calculated by subtracting the value (2+3+6+7)/4 from (1+4+5+8)/4. When this difference is large, the condition being tested is a critical parameter and must be carefully controlled. The sign of the difference indicates the which condition gives the better analytical result (e.g., if the the upper case gives a positive difference, it is the proper experimental condition for the method). If a difference for an experimental condition is small regardless of the sign of the difference, the condition being tested is one that need not be carefully controlled, and small variations from laboratory to laboratory or from analyst to analyst will not significantly affect method performance. The overall method precision (standard deviation) may be estimated from the individual differences calculated during the ruggedness test by taking the square root of 2/7 of the sum of the squares of individual differences (Σ Da + ... Dg). This formula can be recognized as the normal calculation for standard deviation as shown below:

$$\sigma = \sqrt{\frac{\Sigma(X_i - \bar{X})^2}{n - 1}}$$

where:

$$7 = n - 1 \text{ and } 2\ \Sigma\ Da + \ldots Dg = \sum_{i = a}^{g} (X_i - \bar{X})^2$$

Ruggedness Testing of Aqueous Sample Preparation and Analysis Procedures

Table 15 gives the seven experimental conditions that were used in the ruggedness test of Method 8140 for water samples. The recoveries of seven organophosphorous compounds in the eight determinations are presented in Table 16. The seven organophosphates chosen for the water ruggedness test were selected to represent the variety of physical and chemical properties of the full list of method analytes. The recoveries for malathion and ethyl parathion are unacceptably high for several of the determinations. These high recoveries were a result of interference on the packed-column chromatogram, and this demonstrates the limitation of packed column analysis. Because obtaining a recovery of greater than 100 percent is not better than a 100 percent recovery, recovery values greater than 120 were set equal to 100 when the ruggedness test calculations were made. These adjusted values are identified as greater than 100 in Table 16.

The calculated values for each condition (Da ... Dg) and for each compound tested are presented in Table 17. After correcting for high recoveries that were the result of poor chromatography, the individual values are small, $|D_x| < 11$, and the calculated overall-standard derivation for the method is 6.4 percent, for the parameters and conditions included in this test. This is good precision for an environmental method. Therefore, the sample preparation and analysis techniques of Method 8140 are sufficiently rugged for the interlaboratory determination of organophosphorous insecticides in aqueous samples. The results of this study also indicate that sample recoveries are not significantly affected by varying the seven experimental conditions within the ranges given in Table 15.

Ruggedness Testing of Method 8140 Applied to Extraction of Soil

Because of the obvious deficiencies in packed column analysis, the ruggedness tests for soil were conducted by using capillary GC analysis. It was necessary to run two ruggedness tests in order to identify all of the critical parameters

TABLE 15. CONDITIONS VARIED FOR THE RUGGEDNESS TEST OF METHOD 8140 APPLIED TO CLEAN WATER

Parameters to Be Tested	Condition 1	Condition 2
Duration of extraction (Method 3510)	A: 1 minute	a: 0.5 minutes
Amount of sample	B: 1 liter	b: 500 mL
Storage of extracts prior to analysis	C: 3 days	c: 2 weeks
Amount of extraction solvent	D: 60 mL	d: 25 mL
Concentration Method, KD or rotovap	E: KD	e: Rotovap
Injection volume	F: 3 µL	f: 1 µL
Quantification by peak height or area	G: Area	g: peak height

TABLE 16. PERCENT RECOVERIES OF ORGANOPHOSPHOROUS INSECTICIDES FROM WATER USING CONDITIONS OF RUGGEDNESS TABLE

Compound Determination	µg/mL Expected	Determination No.							
		1	2	3	4	5	6	7	8
Chlorpyifos	1.07	107	102	107	111	102	109	101	93
Disulfoton	1.07	104	99	103	103	97	107	99	90
Fenthion	1.05	108	102	107	109	100	111	103	92
Malathion	1.01	>100	102	107	>100	91	>100	112	118
Parathion, ethyl	1.06	101	103	108	>100	104	123	101	97
Phorate	0.92	102	100	103	105	99	108	99	91
Sulfotep	1.03	99	111	113	90	117	117	108	101

TABLE 17. DIFFERENCES FOR RUGGEDNESS TEST IN CLEAN WATER

	Analytical Condition						
	A	B	C	D	E	F	G
Compound	Duration of Extraction	Amount of Sample (H2O)	Storage of Extracts	Amount of Extract Solvent	Concentration Method	Injection Volume	Qualification Area or Height
Chlopyrifos	6	2	0	-6	0	-2	6
Disulfoton	4	2	1	-4	2	-4	6
Fenthion	4	2	1	-6	1	-4	8
Malathion	-3	-11	-3	8	5	-3	-2
Parathion, ethyl	-3	7	-3	-9	5	-9	3
Phorate	3	2	0	-9	0	-3	5
Sulfotep	-8	8	4	-4	1	-10	7
$\bar{X}$	4.4	4.8	1.7	6.6	2	5	5.3
$\bar{X}^2$	19.6	23.6	2.9	43.2	4	25	27.9

Sum $\bar{X}^2 = 146.$

Standard deviation $= [2(94) \div 7]^{.5} = 6.4.$

in soil sample preparation and analysis. The first ruggedness test was conducted by using only the sonicator (Method 3550) with the same 13 compounds used in the soil recovery study. The second ruggedness test was conducted by using both sonication and soxhlet extraction with a larger number of analytes including TEPP, demeton, dimethoate, methyl parathion, and fensulfothion. The 16 analytes used in the second ruggedness test represent the full range of method parameters as far a volatility, polarity, water solubility, and substituent groups.

The seven conditions tested in the first soil ruggedness test are given in Table 18; these were varied according to Youden's experimental design presented in Table 14. The percent recoveries for each compound in each of the eight experiments were determined by using GC analysis with an SPB-608 column and the FPD given in Table 19. The recovery differences were calculated as described above and are presented in Table 20. Because there is too much data to interpret when all of the differences for each compound are considered, the data was reduced by calculating the sum of all compounds tested for each experimental condition. The sum of the differences is also reported in Table 20.

TABLE 18. LEVELS OF CONDITION VARIED IN THE FIRST RUGGEDNESS TEST OF METHOD 8140 APPLIED TO SOIL

Parameters to be Tested	Condition 1		Condition 2	
Sonicator power	A:	7	a:	5
Percent moisture of soil	B:	Dry	b:	17 percent water
Extraction solvent	C:	1:1 acetone/methylene chloride	c:	Methylene chloride
Length of sonication	D:	3 minutes	d:	1 minute
Post extraction filtration method	E:	Gravity	e:	Vacuum
Final concentration step	F:	Blowdown to 1 mL	f:	Blowdown to dryness, redissolve in 1 mL hexane
Clean-up	G:	No GPC	g:	GPC

TABLE 19. PERCENT RECOVERY FOR 13 ORGANOPHOSPHOROUS PARAMETERS OF THE FIRST SOIL RUGGEDNESS TEST

Compound	Experiment							
	1	2	3	4	5	6	7	8
Azinphos methyl	40	61	77	106	75	107	119	55
Chlorpyrifos	110	66	65	76	73	100	113	37
Coumaphos	71	46	62	67	72	94	113	26
Dichlorvos	81	NR	NR	92	74	NR	60	60
Disulfoton	93	NR	NR	45	59	NR	113	16
EPN	123	71	80	84	79	117	100	30
Fenthion	106	95	53	81	75	71	94	46
Malathion	97	51	66	93	75	100	114	36
Parathion, ethyl	106	58	69	86	75	100	114	36
Phorate	92	19	30	72	59	35	54	35
Ronnel	107	60	64	80	70	99	117	30
Sulfotep	96	32	47	75	64	81	97	35
Tetrachlorvinphos	92	87	77	98	80	100	100	53

NR = Not recovered.

TABLE 20. DIFFERENCES FOR THE FIRST SOIL RUGGEDNESS TEST

Compound	Condition						
	A	B	C	D	E	F	G
Azinphos methyl	-18	-18	-4	-22	-20	-22	26
Chlorpyrifos	-2	14	20	8	-4	-12	40
Coumaphos	-15	4	21	-10	-11	-20	30
Dichlorvos	-5	-14	16	9	-21	63	25
Disulfoton	-13	-5	51	30	-27	25	44
EPN	8	24	20	-9	4	13	41
Fenthion	13	6	21	3	-5	11	33
Malathion	-4	4	18	-9	-8	-8	44
Parathion, ethyl	-1	9	21	-4	-5	6	42
Phorate	7	3	19	-13	-3	30	27
Ronnel	-1	11	22	0	-7	-12	45
Sulfotep	-7	5	20	14	-2	3	43
Tetrachlorvinphos	5	8	3	6	-11	10	23
Sum of differences	-33	51	248	3	-120	87	463

Two critical method parameters for the extraction of soil were identified by this first ruggedness test, the solvent choice and whether GPC is used for sample clean-up. Extraction with 1:1 methylene chloride/acetone resulted in improved recoveries over that with methylene chloride alone based on the magnitude of the sum for Condition C. Based on the consistently large positive differences obtained with condition G, it appears that the use of GPC should be avoided. Subsequent discussions with Jim Johnson of the Region 9 laboratory at Las Vegas at EMSL-LV and with Tim Supgeon of ABC Laboratories indicate that many phosphates elute before the analyte collection period of the SW-846 GPC program that was used in this ruggedness test and that small shifts in column calibration can drastically effect sample recovery. In addition, many phosphates are degraded by traces of hydrochloric acid in methylene chloride during GPC cleanup.[32] Therefore, GPC could be used as an option in Method 8140, but only if the laboratory calibrates their GPC apparatus by using organophosphate insecticides rather than the standard phthalate/PCP mixture and only if the laboratory removes any residual hydrochloric acid from its methylene chloride. Of course, when the GPC program for organophosphates requires collection prior to the elution of phthalates, the technique does not remove all high molecular weight interferences from the extract and will therefore not be as useful for sample cleanup. A third method parameter, use of vacuum filtration, significantly increased recovery of azinophos methyl, dichlorvos, and disulfoton, but none of the other analytes. It was not considered to be a critical method parameter. The other conditions being tested in this first soil ruggedness test did not give consistent results. Sonicator power, percent moisture of the soil extracted, length of sonication, and even blowing the sample dryness prior to GC analysis did not affect the calculated differences in a way that consistently increased or decreased recoveries for all of the analytes tested. This lack of consistent variation is probably the result of the variety of physical and chemical properties of the 13 organophosphates tested with this ruggedness test and of the inherent variability of the method.

The second soil ruggedness test repeated two experimental conditions of the first test, GPC cleanup and percent moisture of the soil. It also included five new conditions that are listed in Table 21. A total of 16 analytes were used in the ruggedness test, and analysis was performed using a DB-210 megabore capillary column.

TABLE 21. LEVELS OF CONDITION TO BE VARIED FOR THE SECOND RUGGEDNESS TEST OF METHOD 8140 APPLIED TO SOIL

Parameters to be Tested		Condition 1		Condition 2
Type of extraction	A:	Soxhlet	a:	Sonicate
Percent moisture	B:	Dry	b:	30 percent
Spike level	C:	50 ng/g	c:	500 ng/g
Filtration of extract through sodium sulfate	D:	Filter	d:	Do not filter
Concentration and exchange method	E:	K-D	e:	Rotovap
Length of storage of unconcentrated extract	F:	Concentrate and analyzed immediately	f:	Concentrate and analyze after two weeks
Clean-up	G:	GPC	g:	No GPC

The percent recovery for the 16 organophosphorous analytes determined in the ruggedness test are presented in Table 22; the differences calculated for the ruggedness test are presented in Table 23. Along with the differences for individual compounds, the sum for each condition is also presented in order to simplify the large matrix for each of the 16 compounds and the seven different analytic conditions. As with the first soil ruggedness study, GPC cleanup using the SW-846 phthalate/PCB calibration method significantly reduces recovery of organophosphates. While the sum for the percent moisture shows that samples should be wet, it is a sum of many small numbers, so soil moisture variations between the levels tested here are probably not significant. The other condition varied in the ruggedness test that appears to significantly affect analyte recovery is the spike level (Condition C). A 10-fold increase in spiking concentration resulted in a significant improvement in sample recovery. This is an expected result as recovery normally improves with increased concentration of analyte. Filtration of soil extract through sodium sulfate prior concentration appears to reduce recovery of organophosphate pesticides, especially for dichlorvos, demeton, phorate, and parathion. Therefore, a caution has been added to the proposed method in Appendix A. Finally, the length of storage of unconcentrated extract (Condition F) gave a large negative sum; this indicates that sample extracts should be stored for two weeks prior to concentration. One possible explanation for this result can be deduced from the fact that the largest negative values were observed for dichlorvos, dimethoate, and fensulfothion, the most water soluble of the organophosphate pesticides. This indicates that when samples are concentrated immediately after extraction, the water soluble analytes may be trapped in the thin water film coating the glass of the K-D concentrator or in soil particulates. Such losses have been described by other workers.[32,33,34] This type of loss may be minimized on storage because the analytes partition into the bulk of the methylene chloride during the holding period. While no method can require a two week storage of organic extract prior to concentration and analysis, a caution should be written into Method 8140 to ensure analysts achieve quantitative transfer of the sample extract following concentration steps. The analyst should be advised that analyte losses may result from adsorption of organophosphates onto glassware or particulates carried into the concentration step. Quantitative transfers of extract concentrates can be done most reliably with a hexane/acetone solvent mixture to ensure that both the water and the organic soluble analytes are transferred from the K-D apparatus.

TABLE 22. PERCENT RECOVERY FOR 16 ORGANOPHOSPHOROUS PARAMETERS OF THE SECOND SOIL RUGGEDNESS TEST

Compound	Condition							
	1	2	3	4	5	6	7	8
Azinphos methyl	36	84	123	60	63	60	47	80
Chlorpyrifos	18	77	52	49	65	60	31	76
Coumaphos	41	84	76	48	47	61	37	72
Dichlorvos	9.2	75	46	52	32	56	31	8
Demeton	35	48	60	43	73	38	54	51
Dimethoate	36	90	66	48	NR	68	47	82
Disulfoton	4.9	72	46	48	56	56	NR	76
Fensulfothion	30	87	99	63	NR	63	42	76
Fenthion	47	77	47	51	45	60	16	77
Malathion	55	86	100	59	94	67	65	85
Methyl parathion	17	83	66	59	60	63	9.7	82
Parathion, ethyl	17	80	58	51	66	60	30	78
Phorate	9.6	77	47	50	55	59	30	78
Ronnel	7.3	78	46	50	59	60	26	77
Sulfotep	20	80	53	50	62	61	29	77
TEPP	NR	74	56	51	NR	51	NR	77

NR = Not recovered.

TABLE 23. DIFFERENCES CALCULATED FOR SECOND SOIL FOR RUGGEDNESS TEST

Compound	Condition A	B	C	D	E	F	G
Azinphos methyl	-14	-16	-4	-14	12	-18	-36
Chlorpyrifos	-9	3	-25	-6	-5	-3	-28
Coumaphos	8	0	-4	0	8	-12	-23
Dichlorvos	13	9	-29	-26	-28	-27	-20
Demeton	-19	-1	14	-25	-5	-2	-18
Dimethoate	11	-13	-35	19	39	-27	-9
Disulfoton	-4	5	-36	-13	2	3	-35
Fensulfothion	25	-25	-28	3	19	-31	-16
Fenthion	7	-3	-27	3	11	5	-18
Malation	-3	-1	4	-7	1	-4	-30
Parathion, ethyl	-7	2	-24	-32	-4	-4	-31
Parathion methyl	2	-20	-12	-14	4	-1	-36
Phorate	-10	-1	-31	-26	-5	-5	-27
Ronnel	-11	1	-32	-7	-6	-4	-29
Sulfotep	-6	4	-23	-6	-3	-4	-28
TEPP	13	-15	-49	-1	15	-7	-26
Sum of differences	-4	-71	-341	-190	-47	-141	-410

Based on the results of the two soil ruggedness tests, into improvements in Method 8140 were incorporated into the modified method described in Appendix A. However, because of the overall variation in the differences calculated from the soil ruggedness test, the method precision can not be calculated based on Youden's formula since at least two conditions tested in each test gave large differences.[3] The method precision is available from the soil spiking experiments for the 13 organophosphorous compounds reported in Table 13, as well as from the recovery studies for all 27 analytes reported in the Section 6.

SECTION 6

RESULTS AND DISCUSSION

EXPERIMENTAL DESIGN

The modified Method 8140 presented in Appendix A was validated by S-CUBED in a single laboratory study for 26 of the 27 compounds listed in Table 2. The compounds for which this method has been validated and analyte detection limits in water and soil are presented. Mixtures containing all of the 27 proposed analytes of Method 8140 were spiked into water and were extracted by using Methods 3510 or 3520. The same mixtures were spiked into soil and extracted using Methods 3540 and 3550. This validation was accomplished by spiking samples at three concentrations: 5-15 μg/kg, 50-150 μg/kg, and 500-1500 μg/kg. The range of concentrations reported is a result of the use of 30 g of soil for sonication and 10 g of soil for soxhlet extraction and was calculated based on addition of .150 μg, 1.50 μg, and 15 μg (absolute amount of analyte) to achieve the respective low, medium, and high concentrations. The low and high concentrations were run one time each, and the medium concentrations were determined by five replicate spiked samples. The recoveries were established for each analyte for each of the four extraction methods at all three concentrations. The precision of the method was determined by calculating the standard deviation for the five replicate medium level determinations. All GC analyses were accomplished by using the DB-210 megabore capillary and the FPD.

RESULTS FOR AQUEOUS SAMPLES

The recoveries of the proposed method analytes from water are presented in Table 24 (separatory funnel) and Table 25 (continuous extractor). When use was made of the separatory funnel (Method 3510), the recovery of monocrotophos was less than 20 percent for either method at all three concentrations. Recoveries of chlorpyriphos, dimethoate, fenthion, mevinphos, merphos, naled, trichloronate, and tokuthion were below 20 percent for the low level spike but were greater than 60 percent at either the medium or high concentrations. The recovery of methyl parathion was between 40 and 50 percent, and the recovery of TEPP had an unacceptably high standard deviation (72 percent). The recovery of azinophos methyl and bolstar was greater than 125 percent because of interference. The recoveries of the other compounds tested was between 65 and 125 percent.

The recoveries using continuous liquid extraction (Method 3520) were generally lower than for separatory funnel extraction at low and medium concentrations. This

TABLE 24. RECOVERY OF 27 ORGANOPHOSPHATES BY SEPARATORY FUNNEL EXTRACTION

Compound	Low	Spike Level (µg/L)	Medium	Spike Level (µg/L)	High
Azinphos methyl	126	0.40	143 ± 8	1.50	101
Bolstar	134	0.33	141 ± 8	1.63	101
Chlorpyrifos	7	0.31	89 ± 6	1.56	86
Coumaphos	103	0.31	90 ± 6	1.57	96
Demeton	33	0.41	67 ± 11	2.05	74
Dimethoate	NR	0.32	47 ± 3	1.60	101
Diazinon	136	0.35	121 ± 9.5	1.74	82
Dichlorvos	80	2.85	79 ± 11	14.3	72
Disulfoton	48	0.43	92 ± 7	2.16	84
Ethoprop	82	0.37	90 ± 6	1.83	80
EPN	113	0.45	125 ± 9	1.86	97
Fensulfothion	84	0.41	82 ± 12	2.07	96
Fenthion	NR	0.45	48 ± 10	2.23	89
Malathion	127	0.36	92 ± 6	1.80	86
Merphos	NR	0.34	79	1.71	81
Mevinphos	NR	0.42	NR	2.11	55
Monocrotophos	NR	2.55	18 ± 4	12.7	NR
Naled	NR	0.46	NR	2.29	NR
Parathion, ethyl	101	0.40	94 ± 5	2.02	86
Parathion, methyl	NR	0.38	46 ± 4	1.92	44
Phorate	94	0.42	77 ± 6	2.0	73
Ronnel	67	0.31	97 ± 5	1.57	87
Sulfotep	87	0.40	85 ± 4	1.99	83
TEPP	96	3.91	55 ± 72	19.5	63
Tetrachlorvinphos	79	0.31	90 ± 7	1.54	80
Tokuthion	NR	0.42	45 ± 3	2.08	90
Trichloroate	NR	0.37	35	1.87	94

NR = Not recovered.

High spike level is 10X the medium spike level.

TABLE 25. RECOVERY OF 27 ORGANOPHOSPHATES BY CONTINUOUS LIQUID EXTRACTION

Compound	Low	Spike Level (μg/L)	Medium	Spike Level (μg/L)	High
Azinphos methyl	NR	0.40	129	1.50	122
Bolstar	NR	0.33	126	1.63	128
Chlorpyrifos	13	0.31	82 ± 4	1.56	88
Coumaphos	94	0.31	79 ± 1	1.57	89
Demeton	38	0.41	23 ± 3	2.05	41
Dimethoate	NR	0.32	10 ± 8	1.60	102
Diazinon	NR	0.35	128 ± 37	1.74	118
Dichlorvos	81	2.85	32 ± 1	14.3	74
Disulfoton	94	0.43	69 ± 5	2.16	81
Ethoprop	39	0.37	76 ± 2	1.83	83
EPN	NR	0.45	104 ± 18	1.86	119
Fensulfothion	90	0.41	67 ± 26	2.07	90
Fenthion	8	0.45	32 ± 2	2.23	86
Malathion	105	0.36	87 ± 4	1.80	86
Merphos	NR	0.34	80	1.71	79
Mevinphos	NR	0.42	87	2.11	49
Monocrotophos	NR	2.55	30	12.7	1
Naled	NR	0.46	NR	2.29	74
Parathion, ethyl	106	0.40	81 ± 1	2.02	87
Parathion, methyl	NR	0.38	50 ± 30	1.92	43
Phorate	84	0.42	63 ± 3	2.0	74
Ronnel	82	0.31	83 ± 7	1.57	89
Sulfotep	40	0.40	77 ± 1	1.99	85
TEPP	39	3.91	18 ± 7	19.5	70
Tetrachlorvinphos	56	0.31	70 ± 14	1.54	83
Tokuthion	132	0.42	32 ± 14	2.08	90
Trichloroate	NR	0.37	NR	1.87	21

NR = Not recovered.
High spike level is 10X the medium spike level.

may have been due to hydrolysis of analyte in the longer continuous extraction process which is discussed in Section 3. It is important to remember that this difference was observed in the extraction of clean spiked water. For some environmental samples, the loss of analytes in emulsions formed in the separatory funnel could make continuous liquid extraction the preferred method.

RESULTS FOR SOIL SAMPLES

The recoveries of the proposed method analytes from soil are presented in Table 26 (soxhlet) and Table 27 (sonicator). The compounds were spiked into the soil in an acetone solution immediately prior to extraction. When use was made of the soxhlet method (Method 3540), monocrotophos was not recovered. Chlorpyrifos, dimethoate, fenthion, ronnel, mevinphos, and tokuthion were not recovered at low concentration but had recoveries of 50 percent or greater at medium or high concentration. Naled and methyl parathion were recovered at 40 to 50 percent at only some concentrations. TEPP was recovered at greater than 60 percent only at high concentration. All of the other analytes were recovered at levels between 70 and 111 percent.

The recoveries for sonication (Method 3550) were generally lower than with soxhlet extraction (Method 3540). However, earlier studies indicated little if any difference between the two methods. After the lower sonicator recoveries were calculated, the sonicator was retuned, and the extractions were repeated; the results were no different from those that are presented in Table 27. No explanation is offered for why sonicator did not perform reliably during this test of the method but was not a problem during the ruggedness test. More rigid criteria for evaluating sonicator performance and refinements for the sonicator procedure are now being investigated by Battelle and this laboratory. The recoveries were 50 to 80 percent for most spiked phosphates when the soxhlet extractor was used and only 40 to 70 percent when sonication was used. The relative standard deviation for the method using either soxhlet or sonicator for most analytes is roughly equivalent, in the range of 5 to 15 percent. These data indicate soxhlet extraction may be the most reliable method for the extraction of phosphates from solids.

METHOD PERFORMANCE FOR OTHER PHOSPHATE PESTICIDES

Modified Method 8140 has been used for the analysis of over 100 actual hazardous waste samples under a separate contract with the Industrial Technology Division of EPA. As part of this work, the aqueous recoveries of 15 additional organophosphorous compounds from water were determined. Thirty-six compounds (in two mixtures) were spiked into water at a 50 μg/L level, and recoveries were

TABLE 26. RECOVERY OF 27 ORGANOPHOSPHATES BY SOXHLET EXTRACTION

Compound	Low	Spike Level (μg/kg)	Medium	Spike Level (μg/kg)	High
Azinphos methyl	156	30	110 ± 6	150	87
Bolstar	102	38	103 ± 15	190	79
Chlorpyrifos	NR	31	66 ± 17	156	79
Coumaphos	93	31	89 ± 11	157	90
Demeton	169	41	64 ± 6	206	75
Dimethoate	NR	32	48 ± 7	160	98
Diazinon	87	35	96 ± 3	174	75
Dichlorvos	84	285	39 ± 21	1426	71
Disulfoton	78	43	78 ± 6	216	76
Ethoprop	65	37	70 ± 7	183	75
EPN	114	37	93 ± 8	186	82
Fensulfothion	72	41	81 ± 18	207	111
Fenthion	NR	45	43 ± 7	223	89
Malathion	100	36	81 ± 8	180	81
Merphos	62	34	53	171	60
Mevinphos	NR	42	71	211	63
Monocrotophos	NR	255	NR	1276	NR
Naled	NR	46	48	229	NR
Parathion, ethyl	75	40	80 ± 8	202	80
Parathion, methyl	NR	38	41 ± 3	192	28
Phorate	75	42	77 ± 6	208	78
Ronnel	NR	31	83 ± 12	157	79
Sulfotep	67	40	72 ± 8	199	78
TEPP	36	391	34 ± 33	1954	63
Tetrachlorvinphos	50	31	81 ± 7	154	83
Tokuthion	NR	42	40 ± 6	208	89
Trichloroate	56	37	53	187	53

NR = Not recovered.
High spike level is 10X the medium spike level.

TABLE 27. RECOVERY OF 27 ORGANOPHOSPHATES BY SONICATION

Compound	Low	Spike Level (μg/kg)	Medium	Spike Level (μg/kg)	High
Azinphos methyl	NR	10	27 ± 10	50	21
Bolstar	NR	13	103 ± 15	63	114
Chlorpyrifos	NR	10	79 ± 7	52	77
Coumaphos	NR	10	60	52	15
Demeton	NR	14	NR	69	16
Dimethoate	NR	11	67	53	NR
Diazinon	NR	95	90 ± 14	58	78
Dichlorvos	41	14	13 ± 9	475	27
Disulfoton	30	12	44 ± 22	72	69
Ethoprop	19	12	34 ± 26	61	35
EPN	14	14	86 ± 38	62	105
Fensulfothion	NR	15	37	69	2
Fenthion	NR	12	35	74	84
Malathion	55	12	67	60	31
Merphos	NR	14	71	57	155
Mevinphos	NR	85	NR	70	23
Monocrotophos	NR	15	NR	425	NR
Naled	82	13	40	76	33
Parathion, ethyl	NR	13	74 ± 13	67	75
Parathion, methyl	63	14	NR	64	17
Phorate	NR	10	51 ± 9	69	64
Ronnel	70	13	84 ± 8	52	81
Sulfotep	NR	130	68 ± 10	66	76
TEPP	43	10	7	651	3
Tetrachlorvinphos	NR	14	47 ± 24	51	69
Tokuthion	NR	12	NR	69	82
Trichloroate	NR		NR	62	31

NR = Not recovered.
High spike level is 10X the medium spike level.

determined in five replicate determinations. The extractions were done by using separatory funnel extraction, and the GC analysis was accomplished by using a SPB-608 megabore capillary with an FPD.

The additional 15 compounds of regulatory interest that are not Method 8140 analytes are listed, with retention times on capillary columns, in Table 28. The accuracy and precision of the recoveries of these compounds and of some Method 8140 analytes were measured as the percent recovery of spikes and the relative standard deviation of the replicate determinations. These values are reported in Table 29. The recoveries of these additional compounds are between 60 and 120 percent except for trimethyl phosphate (which is volatile), trichlorfon (which is hydroxylated), and carbofenthion (which co-eluted with DDT in the standard mixture used).

It was found that the addition of more compounds to the analyte list greatly increased the difficulty of identifying and quantitating analytes. It was also found that there were many observed peaks in the chromatograms that did not correspond to the compounds listed in Tables 1 or 28. This was not unexpected as hundreds of organophosphates have been sold and have been disseminated into the environment as agricultural chemicals.[35]

RECOMMENDATIONS

Method 8140 can be used for the analysis of 26 of the 27 analytes listed in Table 1. Recovery for all the compounds except methyl parathion was greater than 65 percent at either the 1 µg/L or the 10 µg/L spike level in water. Recoveries for all compounds except methyl parathion were greater than 60 percent at either the 50 µg/kg or the 500 µg/kg spike level in soil. While methyl parathion recoveries of only 40 and 50 percent were obtained from water and soil in this study, Bill Loy of EPA Region IV reports acceptable recoveries for this compound. The method cannot be used for monocrotophos as it gave recoveries of less than 20 percent.

In addition to the 26 compounds, Method 8140 may be used for many other organophosphorous pesticides, once the accuracy and precision of the recovery of those compounds is established by the analyst. A list of additional compounds for which some recovery data was determined is presented in Table 28.

TABLE 28. RETENTION TIMES FOR ADDITIONAL RCRA COMPOUNDS

Compound	Capillary Column		
	DB5	SPB608	DB210
Additional RCRA Compounds			
Azinphos ethyl	39.04	38.49	37.56
Carbophenothion	–	35.10	33.59
Chlorfenvinphos	33.57	31.86	32.97
Dicrotophos	–	19.12	30.49
Dioxathion	39.93	33.17	37.80
Ethion	36.17	34.79	34.03
Famphur	36.43	35.93	37.86
HMPA	10.69	9.23	–
Leptophos	38.47	37.25	35.51
Phosmet	37.56	37.20	–
Phosphamidon	27.05	24.46	34.07
Terbufos	22.63	18.81	19.54
TOCP	38.79	37.71	–
Trichlorofon	–	25.56	16.73
Trimethylphosphate	2.35	–	–

Temperature Program used for Both Capillary Columns

Initial temperature	50°C
Initial time	1 minute
Rate	5°C/minute
Final temperature	140°C
Hold 1	10 minutes
Rate	10°C/minute
Final temperature	240°C
Hold 2	10 minutes

TABLE 29. RECOVERY OF PHOSPHATE ANALYTES FROM WATER AT 50 μg/L CONCENTRATION

Compound	Percent Recovery	Percent RSD	Analyte of 8140
Azinphos ethyl	77	9	No
Azinphos methyl	83	5	Yes
Carbophenthion	137	16	No
Chlorfenviphos	98	17	No
Chlorpyrophos	84	5	Yes
Coumophos	73	9	Yes
Demeton (oxone)	23	3	Yes
Diazinon	87	5	Yes
Dichlovos (DDVP)	81	5	Yes
Dicrotophos	96	6	No
Dimethoate	43	31	Yes
Dioxathion	80	7	No
Disulfoton	69	5	Yes
EPN	82	6	Yes
Ethion	82	5	No
Famphur	63	15	No
Fensulfothion	67	26	Yes
Fenthion	32	2	Yes
HMPA	120	4	No
Leptophos	77	9	No
Malathion	90	6	Yes
Monocrotophos	9	3	Yes
Naled	74	3	Yes
Parathion, ethyl	83	7	Yes
Parathion, methyl	82	6	Yes
Phorate	97	5	Yes
Phosmet	79	6	No
Phosphamidon	61	16	No
Sulfotep	101	5	Yes
TEPP	82	18	Yes
Terbuphos	87	5	No
Tetrachlorovinphos	88	11	Yes
TOCP	82	8	No
Trichlorofon	40	28	No
Trimethyl phosphate	26	15	No

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APPENDIX A

PROPOSED METHOD 8140: ORGANOPHOSPHOROUS PESTICIDES

1.0 SCOPE AND APPLICATION

1.1 Method 8140 is a gas chromatographic (GC) method used to determine the concentration of various organophosphorous pesticides. Table A1 indicates compounds that may be determined by this method and lists the method detection limit for each compound in reagent water. Table A2 lists the practical quantitation limit (PQL) for other matrices.

1.2 Recoveries for some additional organophosphorous compounds have been determined for water and they include:

Azinphos ethyl
Carbofenthion
Chlorfenvinphos
Dioxathion
Ethion
Famphur
HMPA
Leptophos
Phosmet
Phosphamidion
Terbuphos
TOCP

As Method 8140 has not been fully validated for the determination of these compounds, the analyst must demonstrate recoveries of greater than 70 percent with precision of no more than 15 percent RSD before Method 8140 is used for these or any additional analytes.

1.3 When Method 8140 is used to analyze unfamiliar samples, compound identification should be supported by at least one additional qualitative technique. Section 8.3 provides gas chromatograph/mass spectrometer (GC/MS) criteria appropriate for the qualitative confirmation of compound identifications.

1.4 This method is restricted to use by or under the supervision of analysts experienced in the use of gas chromatography and in the interpretation of chromatograms.

TABLE A1. METHOD 8140 DETECTION LIMITS USING FPD

Compound	Water (3510) (µg/L)	Soil (3540) (µg/kg)
Azinphos methyl	0.10	5.0
Bolstar (Suprophos)	0.07	3.5
Chlorpyrifos	0.07	5.0
Coumaphos	0.20	10.0
Demeton, O,S	0.12	6.0
Dimethoate	0.26	13.0
Diazinon	0.20	10.0
Dichlorvos	0.80	40
Disulfoton	0.07	3.5
Ethoprop	0.20	10.0
EPN	0.04	2.0
Fensulfothion	0.08	4.0
Fenthion	0.08	5.0
Malathion	0.11	5.5
Merphos	0.20	10.
Mevinphos	0.50	25.
Naled	0.50	25.
Parathion, ethyl	0.06	3.0
Parathion, methyl	0.12	6.0
Phorate	0.04	2.0
Ronnel	0.07	3.5
Sulfotep	0.07	3.5
TEPP*	0.80	40
Tetrachlorovinphos	0.80	40
Tokuthion (Protothiofos)*	0.07	5.5
Trichloronate*	0.80	40

*Purity of these standards not established by the EPA Pesticides and Industrial Chemicals Repository, RTP, NC.

TABLE A2. DETERMINATION OF PQL FOR VARIOUS MATRICES[1]

Matrix	Factor[2]
Ground water	10
Low-level soil by sonication with GPC cleanup	670
High-level soil and sludges by sonication	10,000
Non-water miscible waste	100,000

[1] Sample PQLs are highly matrix-dependent. The PQLs listed herein are provided for guidance and may not always be achievable.

[2] PQL = [Method detection limit (Table 1)] x [Factor (Table 2)]. For non-aqueous samples, the factor is on a wet-weight basis.

2.0 SUMMARY OF METHOD

2.1 Method 8140 provides gas chromatographic conditions for the detection of ppb levels of organophosphorous pesticides. Prior to the use of this method, appropriate sample preparation techniques must be chosen. Water samples are extracted at a neutral pH with methylene chloride as a solvent by using a separatory funnel (Method 3510) or a continuous liquid-liquid extractor (Method 3520). Soxhlet extraction (Method 3540) or sonication (Method 3550) using methylene chloride/acetone (1:1) are used for solid samples. Spiked samples are used to verify the applicability of the chosen extraction technique to each new sample type. A gas chromatograph with a flame photometric or nitrogen-phosphorous detector is used for this multiresidue procedure.

2.2 At least five percent of samples (1 in 20) of the same matrix type (solid or liquid) must be spiked in duplicate with an appropriate mixture of standards to determine percent recovery, method precision, and estimated limit of detection for each compound of interest. If samples within a matrix type are greatly different, spikes must be made to each sample type even if more than five percent of the samples are spiked.

2.3 If interferences are encountered in the analysis, Method 8140 may also be performed on extracts that have undergone cleanup using Method 3620 or Method 3660.

3.0 INTERFERENCES

3.1 Method interferences may be caused by contaminants in solvents reagents, glassware, and other sample processing hardware that lead to discrete artifacts or elevated baselines in gas chromatograms. All these materials must be routinely demonstrated to be free from interferences under the conditions of the analysis by running laboratory reagent blanks as described in Section 8.1.

3.2 Refer to Method 3500 (Section 3.5, in particular), 3600, and 8000.

3.3 Florisil, silica gel or "Diol" bonded silica may be used as the cleanup materials for this method. The use of Florisil cleanup materials (Method 3620) for some of the compounds in this method has been demonstrated to yield recoveries less than 85 percent and is therefore not recommended for all compounds. Refer to Table 2 of Method 3620 for recoveries of organophosphorous pesticides as a function of Florisil fractions. The procedure for Diol column cleanup is described in the CLP presticide method. Use of phosphorous- or halogen-specific detectors; however, often obviates the necessity for cleanup for relatively clean sample matrices. If particular circumstances demand the use of an alternative cleanup procedure, the analyst must determine the elution profile and demonstrate that the recovery of each analyte is no less than 85 percent.

3.4 The use of Gel Permeation Chromatography (Method 3640) for sample cleanup has been demonstrated to yield recoveries of less than 85 percent for many method analytes and is therefore not recommended for use with this method.

3.5 A halogen-specific detector (electrolytic conductivity or microcoulometric) is very selective for the halogen-containing pesticides and may be used for the determination of chlorpyrifos, ronnel, coumaphos, tokuthion trichloroate, dichlorvos (DDVP), EPN, naled, and stirophos (tetrachlorvinphos) only.

4.0 APPARATUS AND MATERIALS

4.1 Refer to Methods 3520 to 3540 for necessary glassware for sample preparation.

4.2 Boiling chips: Approximately 10/40 mesh. Heat to 400°C for 30 minutes or Soxhlet extract with methylene chloride overnight.

4.3 Water bath: Heated, with concentric ring cover, capable of temperature control (± 2°C). The bath should be used in a fume hood.

4.4 Balance: Analytical, capable of accurately weighing to the nearest 0.0001 g.

4.5 Sonicator: Heat systems - Ultrasonics, Inc., Model 375C (or equivalent) (375 watt with pulsing capability 1/2-inch or 3/4-inch disruption horn).

4.6 Gas Chromatograph: Analytical system complete with gas chromatograph and all required accessories including syringes, analytical columns, gases, detector and data system, integrator or stripchart recorder. A data system or integrator is recommended for measuring peak areas.

4.6.1 GC Columns

4.6.1.1 Megabore Capillary Columns

Column 1: 15 m x 0.53 mm megabore capillary column, DB-210 (J&W Scientific) or equivalent.

Column 2: 15 m x 0.53 mm megabore capillary column, SPB-608 (Supelco, Inc.) or equivalent.

Column 3: 15 m x 0.53 mm megabore capillary column, DB-5 (J&W Scientific) or equivalent.

4.6.1.2 Packed Columns

Column 4: 8' x 1/4" OD glass column packed with 6% OV-210/4% SP-2100 on Supelcoport 100/120 mesh (or equivalent).

Column 5: 8' x 1/4" OD glass column packed with 5% SP-2401 on Supelcoport 100/120 mesh (or equivalent). A similar column with a three percent loading may be used as well.

4.6.2 Detector: These detectors have proven effective in analysis for all the parameters listed in Section 1.1 and were used to develop the accuracy and precision statements in Section 7.4.6

4.6.2.1 Flame Photometric Detector (FPD) operated in the phosphorous-specific mode is recommended.

4.6.2.2 Nitrogen Phosphorus Detector (NPD)

4.6.2.3 Halogen specific detectors (electrolytic conductivity or microcoulometric) may be used if only halogenated analytes are to be determined.

5.0 REAGENTS

5.1 Reagent water: Reagent water is defined as water in which an interferent is not observed at the method detection limit of each parameter.

5.2 Hexane, acetone, and methylene chloride: Pesticide quality or equivalent.

5.3 Stock standard solutions: Stock standard solutions can be prepared from pure standard materials or can be purchased as certified solutions. Whenever possible, standards must be traceable to primary standards provided by the EPA Pesticides and Industrial Chemicals Repository, RTP, NC. Commercially prepared stock standards can be used if they are verified against EPA standards. If EPA standards are not available for verification then standards certified by the manufacturer and verified against a standard made from pure material is acceptable.

5.3.1 Prepare stock standard solutions by accurately weighing about 0.0100 g of pure material. Dissolve the material in pesticide-quality hexane or other suitable solvent and dilute to known volume in a volumetric flask. If compound purity is certified at 96 percent or greater, the weight can be used without correction to calculate the concentration of the stock standard. Commercially prepared stock standards can be used if they are certified by the manufacturer or by an independent source.

5.3.2 Transfer the stock standard solutions into Teflon-sealed screw-cap bottles. Store at 4°C and protect from light. Stock standard solutions should be checked frequently for signs of degradation or evaporation, especially just prior to preparing calibration standards from them.

5.3.3 Stock standard solutions must be replaced after six months or sooner if comparison with check standards indicates a problem. All stock standards must be stored in a freezer at -15°C.

5.4 Calibration Standards

Calibration standards at a minimum of five concentration levels for each parameter of interest should be prepared through dilution of the stock standards with isooctane. One of the concentration levels should be at a concentration near, but above, the method detection limit. The remaining concentration levels should correspond to the expected range of concentrations found in real samples or should define the working range of the GC. Calibration standards must be replaced after six months, or sooner if comparison with check standards indicates a problem.

5.5 Internal Standards (if internal standard calibration is used)

To use this approach, the analyst must select one or more internal standards that are similar in analytical behavior to the compounds of interest. The analyst must further demonstrate that the measurement of the internal standard is not affected by method or matrix interferences. Because of these limitations, no internal standard can be suggested that is applicable to all samples.

5.5.1 Prepare calibration standards at a minimum of five concentration levels for each parameter of interest as described in Paragraph 5.4.

5.5.2 To each calibration standard, add a known constant amount of one or more internal standards, and dilute to volume with hexane or other suitable solvent.

5.5.3 Analyze each calibration standard according to Section 7.0.

5.6 Surrogate Standards

The analyst should monitor the performance of the extraction, cleanup (when used), and analytical system and the effectiveness of the method in dealing with each sample matrix by spiking each sample, standard, and reagent water blank with one or two surrogates (e.g., organophosphorous pesticides not expected to be present in the sample) recommended to encompass the range of the temperature program used in this method. Deuterated analogs of analytes should not be used as surrogates for gas chromatographic analysis due to co-elution problems.

6.0 SAMPLE COLLECTION, PRESERVATION, AND HANDLING

6.1 Grab samples must be collected in glass containers. Conventional sampling practices should be followed except that the bottle must not be prewashed with sample before collection. Composite groundwater samples should be collected in refrigerated glass containers in accordance with the requirements of the program. Automatic sampling equipment must be free of Tygon and other potential sources of contamination.

6.2 The samples must be iced or refrigerated from the time of collection until extraction. Chemical preservatives such as chloroform should not be used in the field unless more than 24 hours will elapse before delivery to the laboratory. If the samples will not be extracted within 40 hours of collection, the sample should be adjusted to a pH range of 6.0 to 8.0 with sodium hydroxide or sulfuric acid. Prior to addition of acid or base, mark the water meniscus on the side of the sample bottle for later determination of sample volume.

6.3 All samples must be extracted within seven days of collection.

6.4 Extracts are to be refrigerated (4°C) and analyzed within 40 days of extraction.

7.0 PROCEDURES

7.1 Sample Extraction

7.1.1 Water samples must be extracted at a neutral pH with methylene chloride as a solvent by using a separatory funnel (Method 3510) or a continuous liquid-liquid extractor (Method 3520). Soxhlet extraction (Method 3540) or sonication procedures (Method 3550) are used for solid samples with 50:50 methylene chloride/acetone used as the extraction solvent. Spiked samples are used to verify the applicability of the chosen extraction technique to each new sample type.

7.1.2 Single laboratory data indicates that for some matrix types, separatory funnel extraction of water samples and soxhlet extraction of solids give better recoveries.

7.1.3 Single laboratory data indicates that some method analytes are lost during sample drying with sodium sulfate. Analysts are cautioned to use only minimal amounts of sodium sulfate for sample preparation.

7.1.4 Prior to gas chromatographic analysis, the extraction solvent must be exchanged to hexane. The exchange is performed during the K-D procedures listed in all of the extraction methods. The exchange is performed as follows:

7.1.4.1 Following K-D of the methylene chloride extract to 1 mL by using the macro-Snyder column, allow the apparatus to cool and drain for at least 10 minutes.

7.1.4.2 Momentarily remove the Snyder column, add 50 mL of hexane, a new boiling chip, and reattach the macro-Synder column. Concentrate the extract using 1 mL of hexane to prewet the Synder column. Place the K-D apparatus on the water bath so that the concentrator tube is partially immersed in the hot water. Adjust the vertical position of the apparatus and the water temperature, as required, to complete concentration in 5-10 minutes. At the proper rate of distillation the balls of the column will actively chatter, but the chambers will not flood. When the apparatus volume of liquid reaches 1 mL remove the K-D apparatus, and allow it to drain and cool for at least 10 minutes.

7.1.4.3 Remove the Snyder column and rinse the flask and its lower joint into the concentrator tube with 1-2 mL of hexane. A 5-mL syringe is recommended for this operation. Adjust the extract volume to 10 mL. Stopper the concentrator tube and store refrigerated at 4°C if further processing will be performed immediately. If the extract will be stored longer than two days, it should be transferred to a Teflon-sealed screw-cap vial. Proceed with gas chromatographic analysis if further cleanup is not required.

7.1.4.4 The analyst must ensure quantitative transfer of the extract concentrate. Single laboratory data indicates that this transfer is best accomplished with a hexane/acetone solvent mixture.

7.2 The recommended gas chromatographic column and operating conditions for the instrument are as follows:

7.2.1 Megabore Capillary Column Options:

Three megabore capillary columns are included for analysis of organophosphates by this method. Column 1 (DB-210 or equivalent) and Column 2 are recommended if a large number of organophosphorous analytes are to be determined. If the superior resolution offered by Column 1 and Column 2 is not required, Column 3 (D5-5 or equivalent), Column 4 (OV-210/SP-2100) or Column 5 (SP-2401) may be employed.

7.2.1.1 Megabore capillary column DB-210 or equivalent (15 m x 0.53 mm, 1.0 µm film thickness).

- Helium carrier gas at 5 mL/minute.
- Nitrogen makeup gas at appropriate rate for optimum detector performance.
- Column temperature programmed:

Initial temperature:	50°C
Initial time:	1 minute
Rate:	5°C/minute
Final temperature	140°C
Hold 1:	10 minutes
Rate:	10°C/minute
Final temperature	240°C
Hold 2:	10 minutes, or sufficient for last eluting compound

7.2.2 Column 2 Conditions:

Megabore capillary column SPB-608 or equivalent (15 m x 0.53 mm, 1.5 µm film thickness). Analytical conditions identical to Column 1.

7.2.3 Column 3 Conditions:

Megabore capillary column DB-5 or equivalent (15 m x 0.53 m, 1.0 µm film thickness. Analytical conditions identical to Column 1.

7.2.4 Column 4 Conditions:

Supelcoport (100/120 mesh) coated with 6% OV-210/4% SP-2100 in a 8-feet x 1/4-inch O.D. glass column with nitrogen carrier gas at 30 mL/minute.

Column temperature programmed:

Initial Temperature:	130°C
Initial Time:	3 minutes
Program 1 rate:	5°C/minute
Program 1 final temperature:	180°C
Program 1 hold:	10 minutes
Program 2 rate:	2°C/minute
Program 2 final temperature:	250°C
Program 2 hold:	15 minutes, or sufficient for last eluting compound

7.2.5 Column 5 Conditions:

Supelcoport (100/120 mesh) coated with 5% SP-2401 in a 8' x 1/4"-glass column with nitrogen carrier gas at 30 mL/minute. Analytical conditions identical to Column 3.

7.2.6 Retention time for all analytes on each column are presented in Table A3. The analyst should note that several method analytes co-elutes on columns 3-5.

7.3 The analyst must demonstrate reproducibility of ± 10 percent relative standard deviation for the quantitation of standards using the injection technique to be used for sample analysis prior to system calibration or sample analysis.

7.4 Calibration

Refer to Method 8000 for proper calibration techniques. Use Table 1 and especially Table 2 for guidance on selecting the lowest point on the calibration curve.

7.4.1 The procedure for internal and external calibration may be used. Refer to Method 8000 for a description of each of these procedures.

7.4.2 If cleanup is performed on the samples, the analyst should process a series of standards through the cleanup procedure and then analyze the samples by GC. This will confirm elution patterns and the absence of interferents from the reagents.

TABLE A3. RETENTION TIMES FOR METHOD 8140 ANALYTES

Compound	Packed Column 5% SP2401	 6%OV210/ 4%SP2100	Capillary Column DB5	 SPB608	 DB210
Azinphos methyl	35.67	45.91	38.342	38.04	37.24
Bolstar (Suprophos)	25.39	31.96	36.34	35.08	37.55
Chlorpyrifos	14.37	17.58	31.17	26.88	25.18
Coumaphos	42.73	57.00	39.83	38.87	39.47
Demeton, O,S	12.79	13.67	18.31	15.90	17.24
Dimethoate	--	--	20.636	20.18	27.96
Diazinon	10.47	12.80	24.27	20.02	19.68
Dichlorvos	3.88	4.98	9.63	7.91	12.79
Disulfoton	11.27	13.26	23.71	19.96	20.66
Ethoprop	--	--	18.618	16.48	18.67
EPN	34.39	43.07	37.80	36.71	36.74
Famphur*	--	--	36.40	36.93	37.86
Fensulfothion	34.58	42.10	35.85	35.20	36.80
Fenthion	15.26	18.13	38.83	29.45	28.86
Malathion	18.67	20.44	31.72	28.78	32.58
Merphos	19.53	18.3	26.82	21.73	32.44
Mevinphos	8.56	9.72	14.178	12.88	18.44
Monocrotophos	16.36	18.77	20.04	20.11	31.42
Naled	10.09	11.39	19.01	17.40	19.35
Parathion, ethyl	21.49	23.19	31.85	27.62	33.39
Parathion, methyl	18.00	19.16	31.84	23.71	32.17
Phorate	8.86	10.81	19.94	17.52	18.19
Ronnel	13.24	15.84	29.23	22.98	23.19
Sulfotep	10.19	11.41	20.11	18.02	19.58
TEPP	3.05	3.56	6.44	5.12	10.66
Tetrachlorovinphos	24.29	28.21	34.65	32.99	33.68
Tokuthion (Protothiofos)	18.18	24.58	34.67	24.58	39.913
Trichloronate	--	--	32.19	28.41	29.95

*Method 8140 has not been fully validated for famphur.

Initial temperature	130°C	50°C
Initial time	3 minutes	1 minute
Program 1 rate	5°C/minute	5°C/minute
Program 1 final temperature	180°C	140°C
Program 1 hold	10 minutes	10 minutes
Program 2 rate	2°C/minute	10°C/minute
Program 2 final temperature	250°C	240°C
Program 2 hold	15 minutes	10 minutes

7.5 Analysis

7.5.1 For packed columns, inject 2 to 5 µL of the sample by using the solvent flush technique. Smaller volumes (1 µL) may be injected if automatic devices are employed. Record the volume injected to the nearest 0.05 µL and record the resulting peak size in area units.

7.5.2 For megabore capillary columns automatic injections of 1.0 µL are recommended. Hand injections of no more than 2 µL may be used if the analyst demonstrates quantitation precision of ≥ 10 percent relative standard deviation. The solvent flush technique may be used if the amount of solvent is kept at a minimum. Again, record volume injected to the nearest 0.05 µL and record the resulting peak size in area units. Automatic injections of 1.0 µL are recommended.

7.5.3 If the peak area exceeds the linear range of the system, dilute the extract and reanalyze.

7.5.4 If peak detection is prevented by the presence of interferences, further cleanup is required. Before using any cleanup procedure, the analyst must process a series of calibration standards through the procedure to establish elution patterns and to determine recovery of target compounds. The absence of interference from reagents must be demonstrated by routine processing of reagent blanks through the chosen cleanup procedure.

7.5.5 Naled has been reported to be converted to DDVP on some columns by debromination. If this process is demonstrated on the GC system that is used for this analysis, clean the injector and break off several inches of a megabore column or change the glass wool of a packed column prior to analyzing samples. If subsequent injections of naled give DDVP, report naled as DDVP, but, in this instance, both naled and DDVP may not be reported in the same sample.

7.5.6 Examples of chromatograms for organophosphorous pesticides are shown in Figures A1 through A4.

7.5.7 Method detection limits for soil and water are presented in Table A1. Guidance on estimating practical detection limits are presented in Table A2.

7.5.8 GC retention times for method analytes are presented in Table A3.

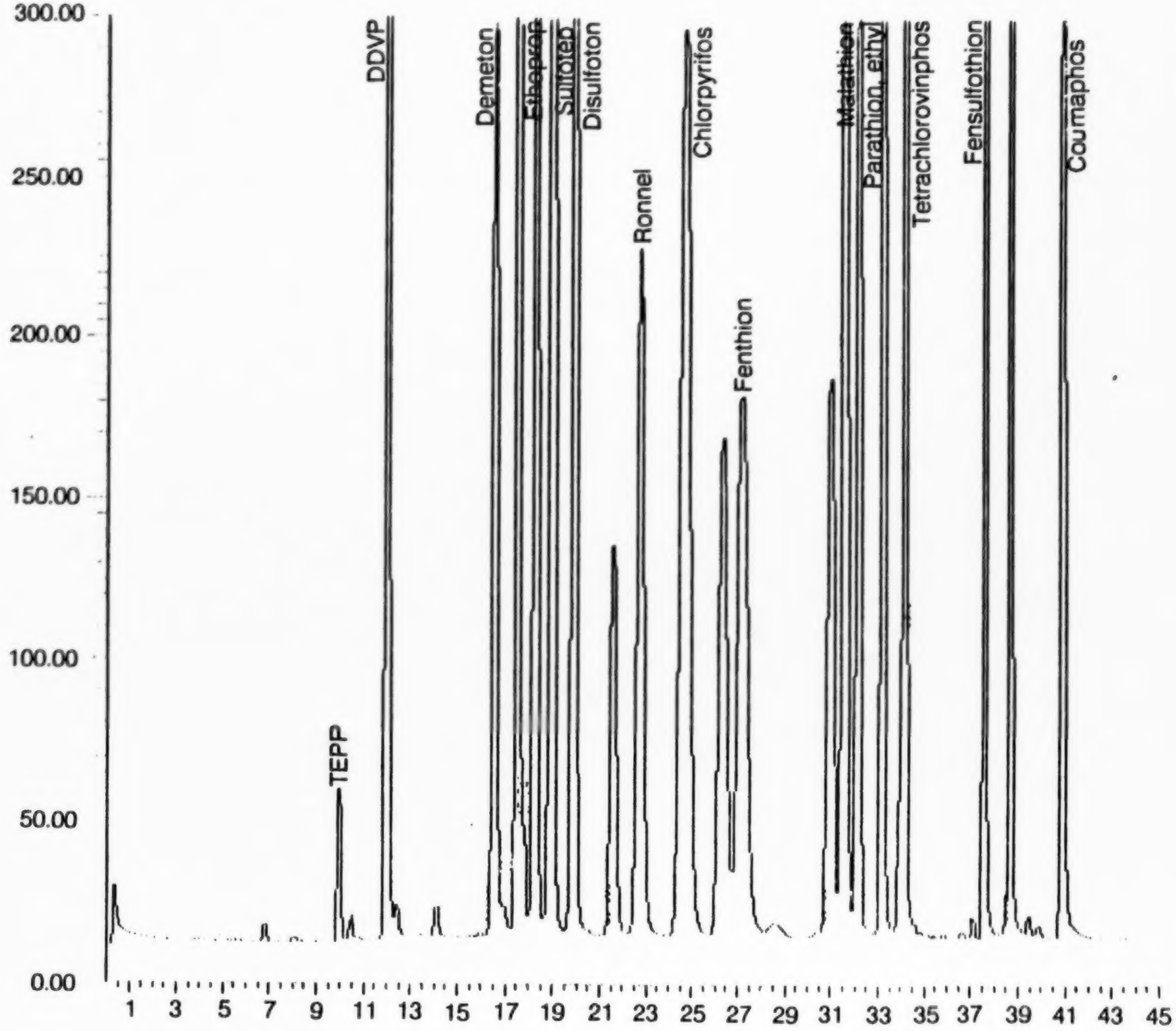

Figure A1. Characteristic response of organophosphates using an FPD detector.

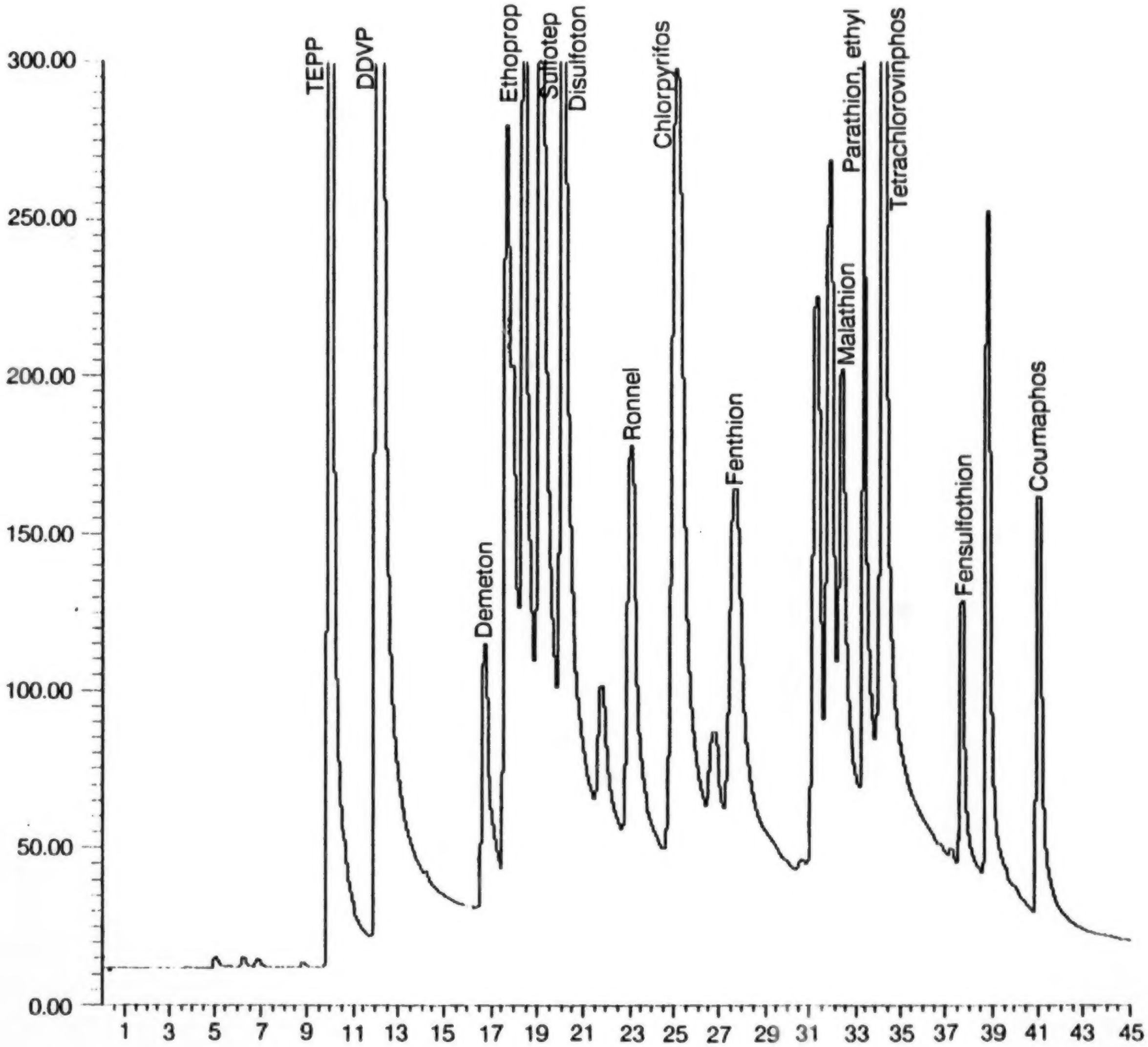

Figure A2. Characteristic response of organophosphates using an NPD detector.

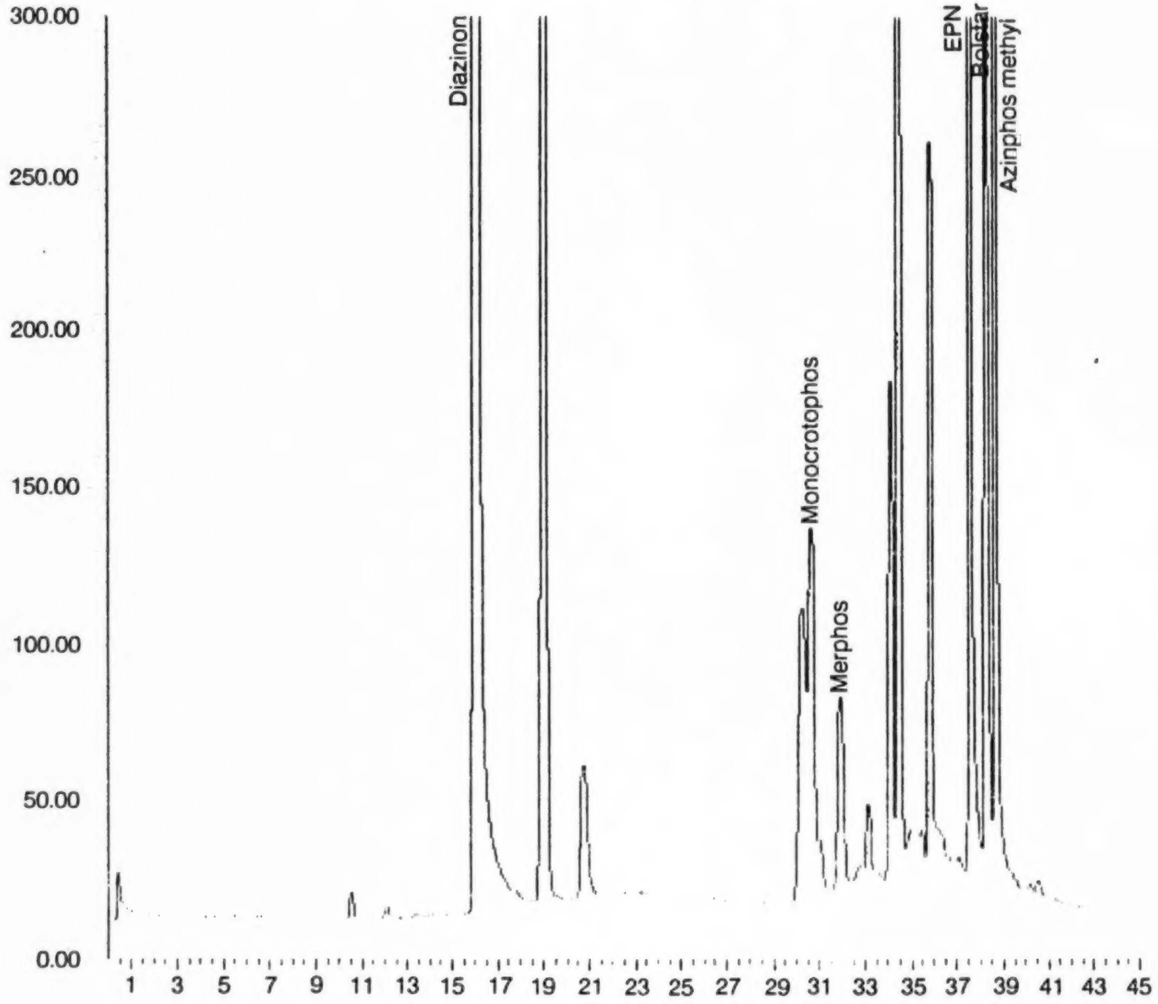

Figure A3. Chromatogram of organophosphates on DB210 with FPD detector.

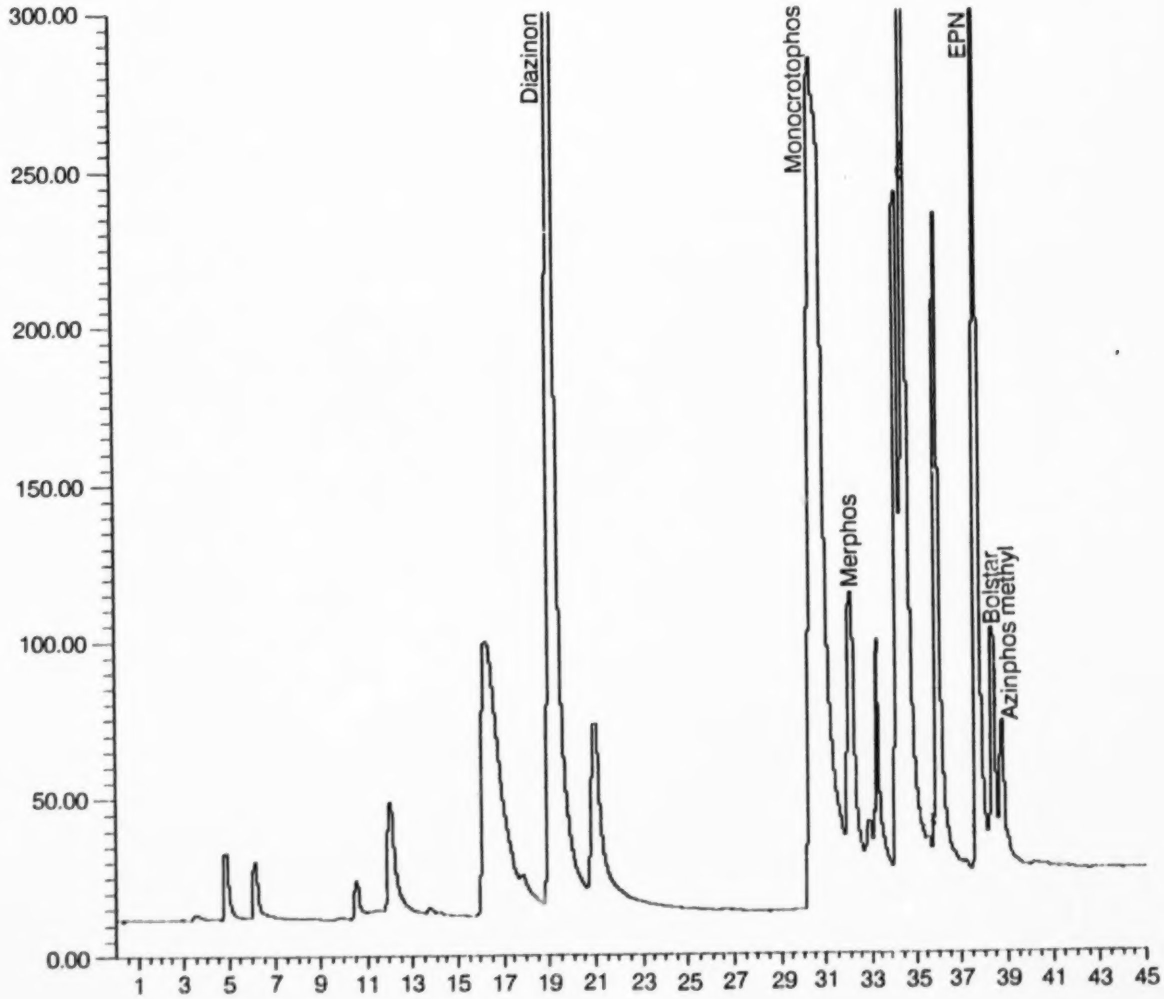

Figure A4. Chromatogram of organophosphates on DB210 with NPD detector.

TABLE A4. RECOVERY OF 27 ORGANOPHOSPHATES BY SEPARATORY FUNNEL EXTRACTION

Compound	Low	Medium	High
Azinphos methyl	126	143 ± 8	101
Bolstar	134	141 ± 8	101
Chlorpyrifos	7	89 ± 6	86
Coumaphos	103	90 ± 6	96
Demeton	33	67 ± 11	74
Dimethoate	NR	47 ± 3	101
Diazinon	136	121 ± 9.5	82
Dichlorvos	80	79 ± 11	72
Disulfoton	48	92 ± 7	84
Ethoprop	82	90 ± 6	80
EPN	113	125 ± 9	97
Fensulfothion	84	82 ± 12	96
Fenthion	NR	48 ± 10	89
Malathion	127	92 ± 6	86
Merphos	NR	79	81
Mevinphos	NR	NR	55
Monocrotophos	NR	18 ± 4	NR
Naled	NR	NR	NR
Parathion, ethyl	101	94 ± 5	86
Parathion, methyl	NR	46 ± 4	44
Phorate	94	77 ± 6	73
Ronnel	67	97 ± 5	87
Sulfotep	87	85 ± 4	83
TEPP	96	55 ± 72	63
Tetrachlorvinphos	79	90 ± 7	80
Tokuthion	NR	45 ± 3	90
Trichloroate	NR	35	94

NR = Not recovered.

TABLE A5. RECOVERY OF 27 ORGANOPHOSPHATES BY CONTINUOUS LIQUID EXTRACTION

Compound	Low	Medium	High
Azinphos methyl	NR	129	122
Bolstar	NR	126	128
Chlorpyrifos	13	82 ± 4	88
Coumaphos	94	79 ± 1	89
Demeton	38	23 ± 3	41
Dimethoate	NR	10 ± 8	102
Diazinon	NR	128 ± 37	118
Dichlorvos	81	32 ± 1	74
Disulfoton	94	69 ± 5	81
Ethoprop	39	76 ± 2	83
EPN	NR	104 ± 18	119
Famphur	–	63 ± 15	–
Fensulfothion	90	67 ± 26	90
Fenthion	8	32 ± 2	86
Malathion	105	87 ± 4	86
Merphos	NR	80	79
Mevinphos	NR	87	49
Monocrotophos	NR	30	1
Naled	NR	NR	74
Parathion, ethyl	106	81 ± 1	87
Parathion, methyl	NR	50 ± 30	43
Phorate	84	63 ± 3	74
Ronnel	82	83 ± 7	89
Sulfotep	40	77 ± 1	85
TEPP	39	18 ± 7	70
Tetrachlorvinphos	56	70 ± 14	83
Tokuthion	132	32 ± 14	90
Trichloroate	NR	NR	21

NR = Not recovered.

TABLE A6. RECOVERY OF 27 ORGANOPHOSPHATES BY SOXHLET EXTRACTION

Compound	Low	Medium	High
Azinphos methyl	156	110 ± 6	87
Bolstar	102	103 ± 15	79
Chlorpyrifos	NR	66 ± 17	79
Coumaphos	93	89 ± 11	90
Demeton	169	64 ± 6	75
Dimethoate	NR	48 ± 7	98
Diazinon	87	96 ± 3	75
Dichlorvos	84	39 ± 21	71
Disulfoton	78	78 ± 6	76
Ethoprop	65	70 ± 7	75
EPN	114	93 ± 8	82
Fensulfothion	72	81 ± 18	111
Fenthion	NR	43 ± 7	89
Malathion	100	81 ± 8	81
Merphos	62	53	60
Mevinphos	NR	71	63
Monocrotophos	NR	NR	NR
Naled	NR	48	NR
Parathion, ethyl	75	80 ± 8	80
Parathion, methyl	NR	41 ± 3	28
Phorate	75	77 ± 6	78
Ronnel	NR	83 ± 12	79
Sulfotep	67	72 ± 8	78
TEPP	36	34 ± 33	63
Tetrachlorvinphos	50	81 ± 7	83
Tokuthion	NR	40 ± 6	89
Trichloroate	56	53	53

NR = Not recovered.

TABLE A7. RECOVERY OF 27 ORGANOPHOSPHATES BY SONICATION

Compound	Low	Medium	High
Azinphos methyl	NR	27 ± 10	21
Bolstar	NR	103 ± 15	114
Chlorpyrifos	NR	79 ± 7	77
Coumaphos	NR	60	15
Demeton	NR	NR	16
Dimethoate	NR	67	NR
Diazinon	NR	90 ± 14	78
Dichlorvos	41	13 ± 9	27
Disulfoton	30	44 ± 22	69
Ethoprop	19	34 ± 26	35
EPN	14	86 ± 38	105
Fensulfothion	NR	37	2
Fenthion	NR	35	84
Malathion	55	67	31
Merphos	NR	71	155
Mevinphos	NR	NR	23
Monocrotophos	NR	NR	NR
Naled	82	40	33
Parathion, ethyl	NR	74 ± 13	75
Parathion, methyl	63	NR	17
Phorate	NR	51 ± 9	64
Ronnel	70	84 ± 8	81
Sulfotep	NR	68 ± 10	76
TEPP	43	7	3
Tetrachlorvinphos	NR	47 ± 24	69
Tokuthion	NR	NR	82
Trichloroate	NR	NR	31

NR = Not recovered.

7.5.9 Single laboratory precision and accuracy using each optional extraction procedure is presented in Table A4 (Method 3510), Table A5 (Method 3520), Table A6 (3540), and Table A7 (Method 3550).

7.6 Analytical Difficulties Encountered with Specific Organophosphorous Pesticides

7.6.1 TEPP

Tetraethylpyrophosphate (TEPP) is an unstable diphosphate which is nearly hydrolyzed in water and is thermally labile (TEPP decomposes at 170°C). Care must be taken to minimize loss during GC analysis and during sample preparation. Identification of bad standard lots is difficult since the EI mass spectrum of TEPP is nearly identical to its major breakdown product, triethylphosphate.

7.6.2 Dichlorvos

The water solubility of dichlorvos is 10 g/L at 20°C and recovery is poor from aqueous solution.

7.6.3 Naled

Naled is converted to dichlorvos on column by debromination. This reaction may also occur during sample workup. The extent of debromination will depend on the nature of the matrix being analyzed. The analyst must consider the potential for debromination when naled is to be determined.

7.6.4 Trichlorofon

Trichlorofon rearranges and is dehydrochlorinated in acidic, neutral, or basic media to form dichlorvos and hydrochloric acid. If this method is to be used for the determination of organophosphates in the presence of trichlorofon, the analyst should be aware of the possibility of rearrangement to dichlorvos to prevent misidentification.

7.6.5 Demeton

Demeton is a mixture of two isomers, α-thiophosphate (Demeton-O) and the corresponding oxon (Demeton-S). Standards for the individual isomers are no longer available through the EPA repository, and two peaks will be observed in all mixed demeton standards. It is recommended that the early eluting oxon (Demeton-S) be used for quantitation.

7.6.6 Merphos

Tributyl phosphorotrithioite is a single component pesticide that is readily oxidized in the environment and during storage to the phosphorotrithioate. The analyst may observe two peaks in the chromatograms of merphos standards.

8.0 QUALITY CONTROL

8.1 Before processing any samples, the analyst should demonstrate through the analysis of a distilled water method blank that all glassware and reagents are interference-free. Each time a set of samples is extracted or there is a change in reagents, a method blank should be processed as a safeguard against laboratory contamination.

8.2 Standard quality assurance practices should be used with this method. Field replicates should be collected to validate the precision of the sampling technique. Laboratory replicates should be analyzed to validate the precision of the analysis. Fortified samples of waste should be analyzed to validate the accuracy of the analysis. Where doubt exists over the identification of a peak on the chromatogram, confirmatory techniques such as mass spectrometry should be used (Section 8.3).

8.3 GC/MS Confirmation

8.3.1 GC/MS techniques should be judiciously employed to support qualitative identifications made with this method. The mass spectrometer should be capable of scanning the mass range from 35 amu to a mass 50 amu above the molecular weight of the compound. The instrument must be capable of scanning the mass range at a rate to produce at least five scans per peak, but not to exceed three seconds per scan. The mass spec is to be operated in the electron impact ionization mode, at 70ev electron energy utilizing 70-V (nominal) electron energy in the electron impact ionization mode. A GC-to-MS interface constructed of all-glass or glass-lined materials is recommended. A computer system that allows the continuous acquisition and storage on machine-readable media of all mass spectra obtained throughout the duration of the chromatographic program should be interfaced to the mass spectrometer.

8.3.2 Gas chromatographic columns and conditions should be selected for optimum separation and performance. The conditions selected must be compatible with standard GC/MS operating practices, such as those described for Method 8250.

8.3.3 At the beginning of each day that confirmatory analyses are to be performed, the GC/MS system must be checked to see that all DFTPP (decafluorotriphenyl phosphine) performance criteria are achieved, as described in Method 8250.

8.3.4 To confirm an identification of a compound, the background-corrected mass spectrum of the compound must be obtained from the sample extract and must be compared with a mass spectrum from a stock or calibration standard analyzed under the same chromatographic conditions. At least 25 ng of material should be injected into the GC/MS. The following criteria must be met for qualitative confirmation:

- The molecular ion and all other ions present above 10 percent relative abundance in the mass spectrum of the standard must be present in the mass spectrum of the sample with agreement to ± 10 percent. For example, if the relative abundance of an ion is 30 percent in the mass spectrum of the standard, the allowable limits for the relative abundance of that ion in the mass spectrum for the sample would be 20 to 40 percent.

- The retention time of the compound in the sample must be within six second of the retention time for the same compound in the standard solution.

- Compounds that have very similar mass spectra can be explicitly identified by GC/MS only on the basis of retention time data.

8.3.5 Where available, chemical ionization mass spectra may be employed to aid in the qualitative identification process because of the extensive fragmentation of OP pesticides during EI MS processes.

8.3.6 Should the MS procedure fail to provide satisfactory results, additional steps may be taken before reanalysis. These steps may include the use of alternate packed or capillary GC columns or additional sample cleanup.

8.4 Method Performance

8.4.1 Estimated detection limits (EDL) and associated chromatographic conditions for water and clean soil are listed above in Table A1. As detection limit will vary with the particular matrix to be analyzed, guidance for estimating PQL's is given in Table A2.

8.4.2 Single operator accuracy and precision studies have been conducted with spiked water and soil samples. The results of these studies are presented in Tables A4-A7.